Rare to be warm in Svalbard:

An ecological and genetic snapshot of four red listed plant species

Master of Science Thesis

Siri Birkeland

Department of Biology

UNIS

CEES

Centre for Ecological and Evolutionary Synthesis

UNIVERSITAS OSLOENSIS MDCCCXI

Supervisor(s): Anne Krag Brysting & Inger Greve Alsos
This work is published digitally through DUO – Digitale Utgivelser ved UiO
http://www.duo.uio.no
It is also catalogued in BIBSYS (http://www.bibsys.no/english)

Abstract

Climate change is emerging as the most far reaching and significant stressor on Arctic biodiversity and it is predicted that it will lead to large changes in distributions, geographical ranges and abundances of species. Many species might soon be at extinction risk, and subsequently will good management of flora and fauna be of outermost importance. In high Arctic Svalbard, a third of the vascular flora is found in the regional red list, but management is difficult due to limited knowledge. For four of Svalbard's most threatened vascular plant species, *Botrychium lunaria*, *Sibbaldia procumbens*, *Kobresia simpliciuscula* ssp. *subholarctica* and *Ranunculus wilanderi* will the following subjects therefore be investigated: 1) Localities, population sizes and possible threats to the populations 2) characteristics of the habitat and dispersal potential within localities 3) levels of genetic diversity and distinctness of the Svalbard populations and 4) implications for conservation in Svalbard.

Evaluation of population sizes and immediate threats was carried out in the field and the data was reported to the Norwegian red list. For the habitat description, a selection of ecological parameters and vegetation data was recorded in 1-2 localities for each of the four focus species. Furthermore, in order to investigate possibilities for population expansion, ecological data was collected both from where the focus species was growing and from sites that the species do not yet occupy in its immediate surroundings. The suitability of this unoccupied habitat was then statistically tested and described through ordinations. Amplified Fragment Length Polymorphism (AFLP) was used to determine levels of genetic diversity, gene flow and genetic distinctiveness of the Svalbard populations compared to selected populations from other parts of the species distribution area.

A thorough mapping of occurrences and population sizes was achieved. New data led to a downgrading of *S. procumbens* and *R. wilanderi* from Critically Endangered to Endangered in the regional red list for Svalbard, while the remaining species were kept in their categories. All populations were restricted to the warmer parts of Svalbard, and although some had local dispersal potential, dispersal potential outside these warm localities is probably low. The level of genetic diversity was extremely low, or nonexistent. Compared to populations from other parts of their distribution range, the Svalbard populations all had the lowest level of genetic diversity observed. *Ranunculus wilanderi*, an endemic for Svalbard, was the only species that seemed to represent an evolutionary divergent line, although data was lacking for *K. simpliciuscula* ssp. *subholarctica*. The focus species all shared an affinity for warmer temperatures, but still climatic induced changes to their habitat can threaten their presence in Svalbard.

Takk til...

Først og fremst vil jeg takke mine fantastiske veiledere, Anne Krag Brysting, Inger Greve Alsos og, om enn ikke på papiret, Reidar Elven. Idunn og jeg er enige i at dere må ha vært det beste veileder-teamet noen sinne. Anne: Du har alltid døren åpen og er ekstremt generøs ovenfor dine litt småforvirrede masterstudenter. Inger: Takk for en super masteroppgave og for mange hyggelige turer i felt. Reidar = guru. Jeg vil også takke Idunn for en flott masterperiode! Vår tur til Grønland og Island er noe jeg sent vil glemme.

Takk til Rune Halvorsen and Lise Heier for å ha vært utrolig hjelpsomme med økologidataene våre. Uten dere hadde det nok blitt et litt mer suspekt resultat.

Nils! Takk for at du reddet meg gang på gang både med GIS og diverse, og for å ha vært utrolig tålmodig under hele masterperioden. Nå skal det ferieres!

En stor takk også til:

Svalbards miljøfond og CEES, Monkeyflower group, Ingrid, alle som har samlet inn materiale, min Sibbaldia-venn Geraldine Allen, min Botrychium-venn Evelyn Williams, Biogjengen, Kristine Westergaard, Det lykkelige kollektivet og selvfølgelig min kjære familie.

Table of Contents

1. Introduction

It is estimated that the current species extinction rate is between 1000 and 10 000 times higher than it would naturally be without human influence (IUCN 2012). The seriousness of this biodiversity loss is now recognized all over the globe, and the United Nations General Assembly has actually declared 2011-2020 the decade of biodiversity. Biodiversity can be considered at many levels of biological variation, ranging from the genetic variability within a species, via the biota of some selected regions of the globe, through the number of evolutionary lineages and the degree of distinctness among them, and finally to the diversity of ecosystems and biomes on Earth (Groom et al. 2006). This master thesis will focus on one globally and three regionally rare plant species and thereby touch several of the biodiversity levels which these species are connected to.

In Svalbard, 54 of the approximately 165 vascular plant species could be found on the regional red list for the archipelago per 2006 (Bakken et al. 2006). There is little knowledge about many of them, even on basics such as localities and population sizes, and this makes good management of the flora difficult. Therefore a project was started up at the University Centre in Svalbard in 2009 in order to provide more detailed information on the most threatened plant species of the archipelago. Four of these species are the ones dealt with here; *Botrychium lunaria, Sibbaldia procumbens, Kobresia simpliciuscula* ssp. *subholarctica* and *Ranunculus wilanderi*.

Svalbard is, like the rest of the Arctic, characterized by some of the largest continuous intact ecosystem on the planet, but is facing increasingly larger threats (Johnsen et al. 2010). Climate change is emerging as the most far reaching and significant stressor on Arctic biodiversity and it is predicted that it will lead to large changes in distributions, geographical ranges and abundances of species (CAFF 2010). In fact, such changes are happening already. Some examples are conversion of tundra to shrubland (Sturm et al. 2005), increasing goose populations with important implications for vegetation (Kery et al. 2006) and a northward movement of the tree line (Serreze 2000). Depending on the magnitude of the change, the resulting Arctic ecosystems may no longer be considered arctic (CAFF 2010). Soon many arctic species might be at extinction risk, and targeting the most extinction prone taxa is urgent.

1.1 Background

Per 2006, *B. lunaria*, *S. procumbens* and *R. wilanderi* were all considered Critically Endangered (CR) in the regional red list of Svalbard, while *K. simpliciuscula* ssp. *subholarctica* was considered Endangered (EN) (Bakken et al. 2006). The latter is the only species that is reported to have several occurrences within the archipelago (Kålås et al. 2010), while the rest have just been reported to occur at one locality each. For *R. wilanderi*, this locality is actually its only known occurrence in the world, being an endemic for Svalbard (Lydersen et al. 2009).

The four focus species also share an affinity for warmer areas. The mutual locality of *B. lunaria* and *S. procumbens* is actually unusually hot for Svalbard, being situated in a geothermal area in Bockfjorden. This locality harbors the highest concentration of red listed species within the archipelago, with four vascular plant species (Elvebakk & Spjelkavik 1981), six bryophyte species (Frisvoll 1978) and one charophyte species (Hoel & Holtedahl 1911) not otherwise known from the islands. The occurrences of *K. simpliciuscula* ssp. *subholarctica* and *R. wilanderi* are also related to areas with relatively favorable climate, as they are located in sheltered fjord areas in Spitsbergen.

It is believed that several of Svalbard's warmth-loving species with small and disjunct present-day populations are partly relicts of larger populations established between 9000 and 4000 years ago (Engelskjon et al. 2003). Indeed, an early Holocene warm period is well documented in a number of proxy records from the Svalbard and western Barents Sea region (Hald 2004). But especially for the species with only one occurrence, recent long distance dispersal might be just as likely. Either way, one might think that as a consequence of global warming, these four species will actually become more common, and that using time on them is wasting limited conservation resources. But this is not necessarily the case.

The response to rising temperature depends on many aspects, like other anthropogenic threats, the genetic condition of the populations, competition from invasive species and other factors apart from temperature that may limit the species distributions. Furthermore, an increase in temperature might come with several additional changes like a decrease of solid precipitation and an increase of mixed precipitation (i.e. sleet), a development that has already been observed at all weather stations in Svalbard (Førland & Hanssen Bauer 2003). The loss of snow cover not only exposes plants to harmful sub-zero ambient temperatures and large temperature fluctuations, but may also lead to damage by winter desiccation, repeated freeze-thaw cycles and abrasion by windblown ice particles (Walker et al. 1999). To assess how

vulnerable these species are to future changes like this in their environment, we therefore need more knowledge on several aspects of their biology.

1.2 What do we need to know to ensure good management of Svalbard's red listed plant species?

A good place to start when collecting information about the four focus species and other red listed species on Svalbard is to pin down their exact localities and population sizes. Without this type of information, it is difficult to place the species in appropriate red list categories. Overestimation, or worse, underestimation of red list categories will seriously confound the process of targeting species in need of conservation. This type of data can also be important for monitoring potential future changes in distribution and population sizes.

Furthermore, predicting the extinction of single populations or species requires ecological and evolutionary information (Lande 1988). An ecological description of the rare species can tell us in what degree their ecological demands are met on Svalbard, and/or how vulnerable their surroundings are to anthropogenic threats like global warming. Limited available habitat or decreasing available habitat will naturally magnify the extinction rate. If there are possibilities for population expansion, a larger population could help buffer the population against future reduction.

Evolutionary processes can further affect extinction rate of populations and/or species in several ways. Threatened species tend to have small isolated populations where inbreeding can greatly reduce the average reproductive individual fitness, and loss of genetic variability from random genetic drift can diminish future adaptability to a changing environment (Frankham 2005; Lande 1988). But according to Lande and Schemske (1985) will the magnitude of inbreeding depression evolve with the mating system. Historically heterozygous outbreeding populations should experience high levels of inbreeding depression, while historically inbred populations should experience lower levels of inbreeding depression. In a historically inbred population will namely recessive deleterious mutations be continually exposed as homozygotes and purged through selection. So when investigating the genetic condition of a population, it is important to take into account these historical factors.

Thorough knowledge about features like mating system is not always available, and might have to be inferred from general genetic structure within the species. A general rule seems to be that outcrossing species often have more of its genetic variation distributed within populations than among populations, while inbreeding species have more variation distributed

among rather than within populations (Loveless & Hamrick 1984). But then again, many other factors tend to influence the genetic structure, like pollination mode, seed dispersal mechanisms, life forms and geographic ranges (CAFF 2010).

Genetics is also an important, (and also the ultimate), component of biodiversity in itself. Maintaining genetic variability is therefore also an important part of fighting biodiversity loss. Evolutionary divergent lines within a species should therefore also be attributed special conservation value (Ryder 1986). This type of information might also be valuable in preventing outbreeding depression (partial reproductive isolation) when other genetic lines are introduced into a population (Frankham 2010).

Finally, to evaluate both natural and man-made threats in the species habitat is important for evaluating extinction rate. If a species is already stressed in its habitat due to natural threats like grazing, competition etc., additional anthropogenic disturbance might just drive it over the edge. Furthermore, identifying the most immediate threats, it will give important clues to appropriate conservation measures.

1.3 The objectives of this study

Together with its mother project, this study will provide information that will be valuable in the management of Svalbard's flora. More specifically, for each of the species *B. lunaria*, *S. procumbens*, *R. wilanderi* and *K. simpliciuscula* ssp. *subholarctica*, the objectives are to:

1. Determine exact localities and population sizes in Svalbard, and give a short evaluation of immediate threats in their current habitat.
2. Give an ecological characterization of the species' habitat within the archipelago, and investigate the possibilities for population expansion in some of the localities mapped in aim 1.
3. Compare levels of genetic variation in the Svalbard populations with reference populations from other parts of their distribution range, and if possible investigate if the Svalbard populations can be an evolutionary divergent unit with special conservation value.
4. Discuss implications for conservation in Svalbard.

Evaluation of population sizes and immediate threats will mainly be carried out in the field. Data for the ecological description will be collected in 1-2 localities for each of the four focus species. Furthermore, in order to investigate possibilities for population expansion, ecological data will have to be collected both from where the focus species is growing and from sites that

the species do not yet occupy in its immediate surroundings. The suitability of this unoccupied habitat will then be statistically tested and described through ordinations. For the genetic part, I will use the high resolution Amplified Fragment Length Polymorphism (AFLP) fingerprinting technique.

2. Material and methods

2.1 Focus area and focus species

2.1.1 Focus area

Svalbard is situated between 74° and 81° latitude North, and 10° and 35° longitude East (The Svalbard Treaty 1920), and covers the three coldest out of five arctic bioclimatic subzones defined by the Circumpolar Arctic Vegetation Map project (Walker et al. 2005). These are the arctic polar desert zone (subzone A), the northern arctic tundra zone (subzone B) and the middle arctic tundra zone (subzone C). All the focus species inhabit localities in the middle arctic tundra zone in Svalbard, which is the warmest of the three with mean July temperatures of 4-6°C (Elvebakk 2005). These localities are also all situated in the western and central parts of Spitsbergen, which has a relatively mild climate due to the North Atlantic current (Jónsdóttir 2007). Today, 65 % of Svalbard's land areas and 87 % of the territorial waters are protected as nature reserves and national parks (Nilsen 2011).

Botrychium lunaria and *S. procumbens* are closely linked to Trollkjeldane (the Troll thermal springs) – the northernmost documented hot springs on land, situated at 79º23'N, 13º26'E in Bockfjorden (Hammer et al. 2005). The water temperature in these springs is moderate (28.3ºC is the highest recorded value), but remains remarkably stable throughout the year (Hammer et al. 2005). Trollkjeldane deposit calcium carbonate in the form of calcite, with the source presumably of the underlying Proterizoic marble of the Generalfjellet formation (Hammer et al. 2005). This precipitation of travertine has led to the formation of travertine terraces, which as Elvebakk and Spjelkavik (1981) puts it "gives the landscape similarities to Japanese or Chinese rice terraces" (Figure 1). Bockfjorden has been a part of the Northwest-Spitsbergen national park since 1973, but is per today not a conservation area in itself.

Figure 1. *Travertine terraces in Bockfjorden*

2.1.2 Focus species

Botrychium lunaria (L.) Sw. (Figure 2) is a moonwort in the family *Ophioglossaceae* and has a circumboreal/polar and a bi-polar distribution (Elven et al. 2007). In Svalbard, it is only reported from Bockfjorden (Elvebakk & Spjelkavik 1981), where it was first discovered in 1974. This is the northernmost documented locality of the species. Its habitat is usually dry or moderately damp, open or slightly shady sites on sandy or other light, nutrient poor soil with low or patchy plant cover (Jonsell & Karlsson 2000). Outside Svalbard it often occurs in manmade grassland and is now in rapidly decline due to overgrowth and artificial manuring (Jonsell & Karlsson 2000). Being a pteridophyte, *B. lunaria* has two different life stages: The subterranean perennial gametophyte and the aboveground (also perennial) sporophyte (Farrar 2006). The gametophyte is chlorophyll-free, but with endotrophic mycorrhiza (Jonsell & Karlsson 2000). Reproduction in *Botrychium* usually occurs by union of gametes from the same gametophyte (intragametophytic self-fertilization) since gametes are hindered to swim very far by the soil (Farrar 2006). The base number of chromosomes in *Botrychium* subgenus *Botrychium* is 45 (Farrar 2006) and *B. lunaria* has 2n = 90 and is diploid (Hauk & Haufler 1999).

Sibbaldia procumbens L.(Figure 2) is a small perennial herb in the *Rosaceae* family (Elven et al. 2007). The species has a circumpolar-alpine distribution, but with a large gap occurring in northern Asia (Elven et al. 2007). In Svalbard, it is only found in Bockfjorden, where it was first discovered in 1960 (Rønning 1961). As for *B. lunaria*, this is also the northernmost documented locality for *S. procumbens* with 73° north in east Greenland being the subsequent northernmost locality (Rønning 1961). The plant usually grows in moist gravelly herbmats where snow remains late (Jonsell & Karlsson 2000). Its small inconspicuous, hermaphroditic flowers are insect pollinated elsewhere (Coker 1966), but assumed to be mainly selfing in Svalbard (Brochmann & Steen 1999). *Sibbaldia procumbens* is categorized as a plant with mainly a mixed mode of reproduction (Brochmann & Steen 1999). It is a diploid with 2n = 14 (Brochmann & Steen 1999).

Kobresia simpliciuscula (Wahlenb.) Mack. ssp. *subholarctica* T. V. Egorova (Figure 3) is a perennial herb of 5-30 cm in the *Cyperaceae* family (Elven et al. 2007). *Kobresia simpliciuscula* ssp. *subholarctica* is superficially very similar to subsp. *simpliciuscula*, but the differential characters in inflorescence are deemed to be taxonomically important (Reidar Elven, pers. comm. 2011). *Kobresia simpliciuscula* ssp. *subholarctica* has a circumpolar, but

scattered, distribution (Elven et al. 2007). The subspecies is reported from 7-8 localities in Svalbard (Bakken et al. 2006; Elvebakk 1993). It grows in tussocks on alkaline tundra and marshes (Elven et al. 2007). The flowers are monoecious, with male flowers situated in the top of the inflorescence and female flowers at the bottom (Elven et al. 2007). Main pollination vector is wind (Brochmann & Steen 1999). The subspecies has 2n = 76 chromosomes.

Ranunculus wilanderi (Nath.) Á. Löve & D. Löve (Figure 3) is a perennial herb in the *Ranunculaceae* family. Its only known occurrence on a world basis is Kapp Thordsen, and it is therefore considered an endemic for the archipelago. The habitat is reported to be a damp and deep moss tundra (Elvebakk & Prestrud 1996). *Ranunculus wilanderi* is just one of numerous microspecies within the *Ranunculus auricomus* complex. All members of this complex possess the ability to produce seeds asexually by agamospermy (Jonsell & Karlsson 2000). The species is a tetraploid with a chromosome number of 32 (Brochmann & Steen 1999).

2.2 Mapping of localities and population sizes in Svalbard

Coordinates were taken in all visited localities for the four focus species. If the species was distributed over a larger area, coordinates were taken at the edges to mark the extent. To estimate population sizes I either counted all visible individuals, or extrapolated the total population size from the number of individuals counted in a smaller area (when the number of individuals was large). Flags were put out to mark the extension of the populations and then photographed for future comparison. An evaluation of immediate threats in the locality was done in the field. Possible threats as grazing, fragile landscape, frost disturbance etc. were noted down. The area around the hot springs in Bockfjorden was thoroughly investigated during a three day visit from 31st of July to the 3rd of August 2009. The other locations, Kapp Thordsen, Gipsvika and Ossian Sarsfjellet, were visited one day each in June and July 2009. The results from this part of the study were used for the revision of the red list (Kålås et al. 2010).

2.3 Ecological investigations

To describe the habitat and evaluate possibilities for local expansion, ecological investigations were carried out for each species in the focus area, Svalbard. For *B. lunaria*, *S. procumbens*

and *R. wilanderi*, we sampled ecological data from their only known locality within the archipelago, whereas for *K. simpliciuscula* ssp. *subholarctica*, two localities were investigated (Table 1).

2.3.1 Sampling design

Our sampling unit was frames of 0.5 m x 0.5 m that were laid out in the area containing the species of interest. The frames (or plots) were put down selectively where the focus species was growing, but spread out so that the variation in the area could be captured. The plots were used to investigate if sites occupied by the focus species, shared any unique ecological attributes. To check if these hypothetical ecological attributes were patchily distributed and if the focus species had possibility for expansion within the area we included control plots that did not contain the focus species. A control plot was taken approximately half a meter away from each focus species plot, by rolling the frame in a random direction (where the focus species did not occur). I therefore have plot pairs consisting of a focus species plot and a control plot. Each of the plots contained a grid, dividing the frame in 25 equally sized squares that were used for the vegetation analysis. The study design could to a certain degree be said to be "randomization within selected blocks" (Økland 2007). For an overview of the number of sample units per species and locality, see Table 1.

2.3.2 Frame analysis and recording of explanatory variables

The species composition in each plot was recorded by putting down a knitting needle in each of the 25 crosses in the frame (the point intercept method; Bråthen (2009)). At each cross, the species that touched the needle were assigned a score of 1. Species that were present in the plot, but not registered by the point intercept technique were given a total score of 0.75 if more than one individual were present, and a score of 0.5 if just one individual was present. Only vascular plant species were recorded. In addition to the species composition, a set of ten biotic and abiotic explanatory variables was recorded for each plot. I recorded the percentage cover of vascular plants, cryptogamic crust, bryophytes, lichen, bare ground and stones. The estimation was done by eye, using the grid (partitioned in 25 squares) as a reference. Furthermore, soil temperature, moisture level, slope, aspect, and pH were measured in each plot. The temperature was measured with a digital thermometer (model TFX410, Ebro, Ingolstadt) at 3 cm and10 cm soil depth with three and four replicates respectively. When the focus species was present in the plot, these measurements were taken its vicinity. Temperature

measurements for each site were always performed the same day, within a time span of maximum 1-2 hours. The recorded temperatures were only used to compare plots within localities, because different conditions during measurements (weather conditions, time of day and time of year) will make between-locality comparison impossible. Moisture level was measured by the "finger test" on a rough scale from 1-4, following Raup (1969). The four categories were: (1) dry, (2) moist, (3) wringing wet and (4) dripping wet. The fifth category suggested by Raup (1969; free water standing or running over the surface of the soil) was merged with the fourth category. Slope and aspect were both measured with a compass. To measure pH, a soil sample was taken from each plot, and brought back to the University Centre in Svalbard. In the lab, 4 g soil sample was mixed with 10 ml deionized water, shaken for one hour (180 strokes per minute in a Stuart reciprocating shaker model SSL2, Staffordshire) and left over night to settle. The pH measurements were done with an electronic pH-meter (model MX300 X-mate pro, Mettler Toledo, Zurich) with an accuracy of ± 0.1. Some explanatory variables (slope, aspect, temperature at 10 cm, percentage cover of stones and percentage cover of bare ground) were not recorded for the *K. simplicuiscula* ssp. *subholarctica* site at Gipsvika. In addition, some variables (pH, temperature at 10 cm, cryptogamic crust, percentage of stones and percentage of ground) were missing for a 1-2 plots each in the *K. simpliciuscula* ssp. s*ubholarctica* locality at Ossian Sarsfjellet.

2.3.3 Statistical and descriptive analyses of the ecological data

The minimum, maximum and mean of all explanatory variables were calculated for each focus species. The same calculations were done for the control plots of each species and site as a comparison. Missing values for *K. simpliciuscula* ssp. *subholarctica* were replaced with the mean of the variable for each plot type. The aspect was originally recorded in degrees, but was transformed to a scale where 22.5° (north-northeast) equals zero and 202.5° (south-southwest) equals 180° which is the highest value. As south-southwest is reckoned as the most favorable (or warmest) aspect, we wanted this to be reflected in the scale range. The transformation followed these formulas from Qian (2009):

$$x\ e\ [22.5,202.5]: \qquad y = x - 22.5$$

$$x\ e\ [202.5,360]: \qquad y = 382.5 - x$$

$$x\ e\ [0,22.5]: \qquad y = 22.5 - x$$

Here, x is the original recorded value. To test for differences between the plots with the focus species and the control plots without the species, we performed a Wilcoxon rank sum test between all plot pairs for each species using R version 2.13.0. Missing values were treated as NA. The plot pair differences were checked manually to decide whether a plot type had significantly higher or lower values. To further investigate the differences between the two plot types, and possibly also relate these differences to the explanatory variables, ordinations were used to order the sampling units (the plots) along axes of variation in species composition (Eilertsen 1990). Ordinations were done for each of the focus species, and also for the two sites of *K. simpliciuscula* ssp. *subholarctica* separately. In addition ordinations were done for the whole area around the Troll springs in Bockfjorden. This was done in collaboration with a twin project by Idunn Elisabeth Borgen Skjetne at the University in Oslo, where plot data was collected in the exact same way as in this study. Together we had 60 plots covering the whole area (not shown in Table 1). The main goal was to investigate the focus species in relation to ecological gradients around the Troll springs and their potential for spreading not just within immediate vicinity, but within the whole Troll spring area. Two ordination techniques, DCA (Detrended Correspondence Analysis (Hill & Gauch 1980)) and GNMDS (Global Non-metric Multidimensional Scaling; Kruskal (1964)) were applied in parallel to the plot data in order to enhance the probability of reaching a reliable gradient structure (Økland & Eilertsen 1996). Detrended Correspondence Analysis is an eigenanalysis-based ordination technique, while Global Non-metric Multidimensional Scaling is a distance-based ordination technique. These two ordination techniques are the most popular forms of indirect gradient analysis, mainly because they inhabit rather different strengths and weaknesses (Palmer 2012). Prior to the ordinations, the species data collected from each plot was transformed to a different scale. The chi-square measure of dissimilarity used in DCA is strongly influenced by species with low abundance and low frequency (low totals) in the data set and a down weighting technique recommended by Eilertsen (1990) was used to deal with this problem. The original species data measured on a scale of 0.5-25 was transformed to a new scale of 1-5 with the following formula:

$$y = 1.33(x^{0.411})$$

Here, the new value is y, and x is the old value. The explanatory variables were not used in the ordination itself, but overlaid on the final ordination plots in order to explain the gradients in species composition. All explanatory variables were transformed prior to the ordinations in

order to reduce skewness of their frequency distributions and improve their homoscedasticity following Økland and Økland (2001). One of two formulas was applied to each explanatory variable according to whether the original frequency distribution was left-skewed or right-skewed:

$$y = e^{cx}$$

$$y = \ln(c + x)$$

Here, y is the transformed explanatory variable, x is the original variable and c is a variable that was manually tuned to reduce the skewness. The resulting values for each explanatory variable were then ordered on a new scale from 0-1, with the following formula:

$$\mathrm{z} = \frac{y - y_{min}}{y_{max} - y_{min}}$$

Here, z is the new value on the 0-1 scale, and y values from the previous transformation. Missing values were replaced with the mean of the corresponding plot types at the appropriate locality, prior to all transformations. All data editing and transformations were performed in Microsoft excel 2010. To check for correlations between the explanatory variables, Kendall's τ was calculated in the R version 2.13.0. DCA and GNMDS ordinations were also performed in the R version 2.13.0, but with the vegan package (Oksanen 2011), the MASS package (Venables & Ripley 2002) and the stats package (R Development Core Team 2011). The DCAs were run with detrending by segments and non-linear rescaling, while the GNMDSs were run with the following options: distance measure = Bray-Curtis distance, initial configuration = 100, maximum iterations 100 000 and tolerance = 1e-7. Since no unique best solution exists for a GNMDS, the number of dimensions was chosen on the basis of the maximum number of dimensions where all axes could be correlated to one of the DCA axes in the twin ordination. The configuration with the least stress was chosen among all configurations with the same k (dimensions). To check if the GNMDS and the DCA gave the same results, we used Kendall's correlation coefficient, τ, to measure the correlation between all pairs of GNMDS and DCA axes. A τ of 0.4 was used as a lower limit of acceptable correlation (Liu et al. 2011). One outlier was removed from the Bockfjorden ordinations, and two outliers from the *S. procumbens* ordinations. If DCA ordination results were confirmed by a GNMDS, explanatory variables were overlaid as vectors on the DCA to explain potential differences in species composition. Only DCA-axes 1 and 2 were used when investigating gradients in the DCA, since the first two axes are the only axes that are environmentally

interpretable (Økland & Edvardsen 2006). Correlations between the explanatory variables and the two first DCA axes, were calculated with Kendall's correlation coefficient τ. Finally, we also had to find a way around the problem that an obvious difference between the plots with and without the focus species is the actual presence of the focus species. Therefore all ordinations were also run without the focus species in the species input matrix to check if potential differences were not just a result of this.

2.4 Genetic investigations

2.4.1 Sampling

For *B. lunaria*, *S. procumbens* and *R. wilanderi* material for the genetic analyses was collected from all known localities in the focus area, Svalbard. For *K. simpliciuscula* ssp. *subholarctica*, four of the 7-8 known Svalbard localities were sampled for genetic analyses. In addition, material was also collected from reference populations in other parts of the species' distribution range. A closely related species for each of the four focus species was also sampled to serve as outgroups. For an overview of the genetic sampling, see Table 1 and Figure 2-3. As far as it was possible, leaves from ten individuals situated 5-10 m apart, were collected from each locality. Since we also wanted to investigate if the Svalbard populations might be an evolutionary divergent line, reference populations with a smaller sample size was also included to serve as a genotype reference. Only fresh, healthy leaves were collected, and these were immediately stored on silica to ensure good quality DNA. During sampling on Svalbard, great care was taken not to damage the few populations that exist there. If the population size was really small, only leaves from a few individuals were removed.

Table 1. *Sampled populations of the four focus species. UTM coordinate for each population is given when available. Plots = number of focus species plots/number of control plots. Further Nei's D, % of polymorphic loci, and the frequency-downweighted marker value is given for each population.*

Species	Pop ID	Locality	Collector(s)*	UTM Zone	UTM East	UTM North	Plots (n)	DNA samples (n)	D	Polymorphic loci (%)	DW
Botrychium lunaria	BL01	Bockfjorden, Svalbard (Norway)	IEBS, IGA, RE, SB	33 X	467919	8813750	4/4	3	0.000	0.0	91.77
	BL02	Tasiilaq, Greenland (Denmark)	IEBS, SB	24 W	563545	7277330		7	0.038	10.3	54.68
	BL03	Laugarvatn, Iceland	IEBS, SB	27 W	511095	7120818		2	0.013	1.3	36.08
	BL04	Geysir, Iceland	IEBS, SB	27 W	533711	7132163		2	0.077	7.7	174.06
	BL05	Tønsvikdalen, Norway	TA	34 W	431962	7734124		9	0.087	20.5	64.69
	BL06	Lungau, Austria	AT	33 T	377227	5224128		10	0.102	32.1	95.63
	BL07	Piemonte, Italy	AT	32 T	402676	4890611		10	0.083	25.6	118.76
	BL08	Folldal, Norway	RE, SB	32 V	551736	6890335		10	0.086	21.8	67.46
	BL09	Finse, Norway	IEBS, SB	32 V	-	-		10	0.063	21.8	100.82
	BL10	Reykjanes peninsula, Iceland	RE	27 V	-	-		10	0.019	7.7	40.16
	BL11	Strendur, The Faroe Islands (Denmark)	IEBS, JD	29 V	616389	6886931		10	0.048	19.2	67.43
	BL12	Abruzzo, Italy	PK	33 T	415538	4628070		10	0.100	37.2	123.91
	BL13	Bern, Switzerland	PK	32 T	416894	5167122		10	0.093	30.8	184.03
	BL14	Kåfjord, Norway	RE	34 W	502000	7697400		5	0.087	19.2	78.27
	BL15	Skaftafell, Iceland	ÓBM	28 W	-	-		3	0.043	6.4	77.23
Botrychium boreale	BB02	Folldal, Norway	IEBS, RE	32 V	551301	6882112		5	0.049	10.3	922.33
Kobresia simpliciuscula ssp. *subholarctica*	KS01	Gipsvika, Svalbard (Norway)	IEBS, IGA, SB	33 X	534300	8709400	6/6	9	0.000	0.0	64.25
	KS02	Ossian Sarsfjellet, Svalbard (Norway)	IEBS, IGA, ÓBM, RE, SB	33 X	445560	8763184	5/5	10	0.004	1.8	69.46
	KS03	Blomstrandhalvøya, Svalbard (Norway)	IEBS, IGA, ÓBM, RE, SB	33 X	439900	8768500		10	0.000	0.0	64.61

	KS04	Flatøyrdalen, Svalbard (Norway)	AKB, IGA, PBE, RE	33 X	521345	8802052		9	0.002	0.9	68.52
Kobresia simpliciuscula	KS05	Røros, Norway	RE	32 V	626000	6943600		5	0.027	5.4	123.45
ssp. *simpliciuscula*	KS06	Folldal, Norway	AKB, IEBS, RE, SB	32 V	544218	6909844		8	0.005	1.8	141.36
	KS07	Lungau, Austria	AT	33 T	376830	5224763		3	0.024	3.6	1052.2
Kobresia myosuroides	KM02	Geysir, Iceland	IEBS, SB	27 W	533711	7132163		5	0.058	11.7	1200.5
Ranunculus wilanderi	RW01*	Kapp Thordsen 2009, Svalbard (Norway)	IEBS, IGA, ÓBM, RE, SB	33 X	512179	8709690	5/5	11	0.001	0.6	NA
	RW02*	Kapp Thordsen 2008, Svalbard (Norway)	IGA	33 X	511748	8709654		8			
Ranunculus auricomus	RA01	Folldal, Norway	AKB, IEBS, RE, SB	32 V	553690	6867751		5	0.021	4.1	NA
Sibbaldia procumbens	SP01	Bockfjorden, Svalbard (Norway)	IEBS, IGA, RE, SB	33 X	467857	8814010	3/3	10	0.000	0.0	103.7
	SP02	Bockfjorden, Svalbard (Norway)	IEBS, IGA, RE, SB	33 X	467926	8813458	6/6	10			
	SP03	Bockfjorden, Svalbard (Norway)	IEBS, IGA, RE, SB	33 X	-	-		5			
	SP04	Tasiilaq, Greenland (Denmark)	IEBS, SB	24 W	561680	7278199		10	0.038	11.7	96.1
	SP05	Kulusuk, Greenland (Denmark)	IEBS, SB	24 W	-	-		4	0.046	9.0	96.4
	SP06	Skaftafell, Iceland	ÓBM	28 W	-	-		11	0.006	3.4	97.0
	SP07	Lungau, Austria	AT, KM	33 T	376654	5223929		10	0.010	3.4	109.2
	SP08	Aoste, Italy	AT	32 T	395744	5088415		10	0.008	2.1	94.7
	SP09	Piemonte, Italy	AT	32 T	340588	4949217		10	0.018	8.3	113.3
	SP10	Folldal, Norway	AKB, IEBS, RE, SB	32 V	543923	6909225		10	0.027	6.9	96.2
	SP11	Finse, Norway	IEBS, SB	32 V	-	-		10	0.029	9.0	99.0
	SP12	Vestfirðir, Iceland	RE	27 W	-	-		10	0.041	11.0	104.7
	SP13	Strendur, The Faroe Islands (Denmark)	IEBS, JD	29 V	615676	6890006		8	0.022	5.5	101.0

	SP14	Valais, Switzerland	PK	32 T	428688	5139589	10	0.011	4.1	173.9
	SP15	Nunavut, Canada	BA	12 W	543224	7432152	1	NA		NA
	SP16	Blasendalen Valley, Greenland (Denmark)	KW	22 W	403941	7755516	4	0.102	19.3	183.5
	SP17	Komi, Russia	IGA, AT	41 V	627747	6732980	5	0.043	9.7	411.5
	SP19	Unalaska island, USA	BK	3 U	409023	5972138	5	0.037	8.3	155.5
	SP20	Yukon, Canada	BB	8 W	434229	7532548	5	0.028	6.2	102.6
	SP21	Jan Mayen (Norway)	GA	29 W	-	-	3	0.014	2.1	93.5
Sibbaldia cuneata	SC01	Tromsø Botanical Garden	RE	-	-	-	2	0.021	2.1	1823.1

AKB = Anne Krag Brysting, AT = Andreas Tribsch, BA = Brian Apland, BB = Bruce Bennett, BK = Brad Krieckhaus, GA = Geir Arnesen, IEBS = Idunn Elisabeth Borgen Skjetne, IGA = Inger Greve Alsos, JD = Jan Djurhuus, KM = Karin Moosbrugger, KW = Kristine Westergaard, ÓBM = Ólöf Birna Magnúsdóttir, PBE = Pernille Bronken Eidesen, PK = Patrick Kuss, RE = Reidar Elven, SB = Siri Birkeland, TA = Torbjørn Alm

*NA = No available data, * = treated as one population within each species, Outgroup indicated in bold*

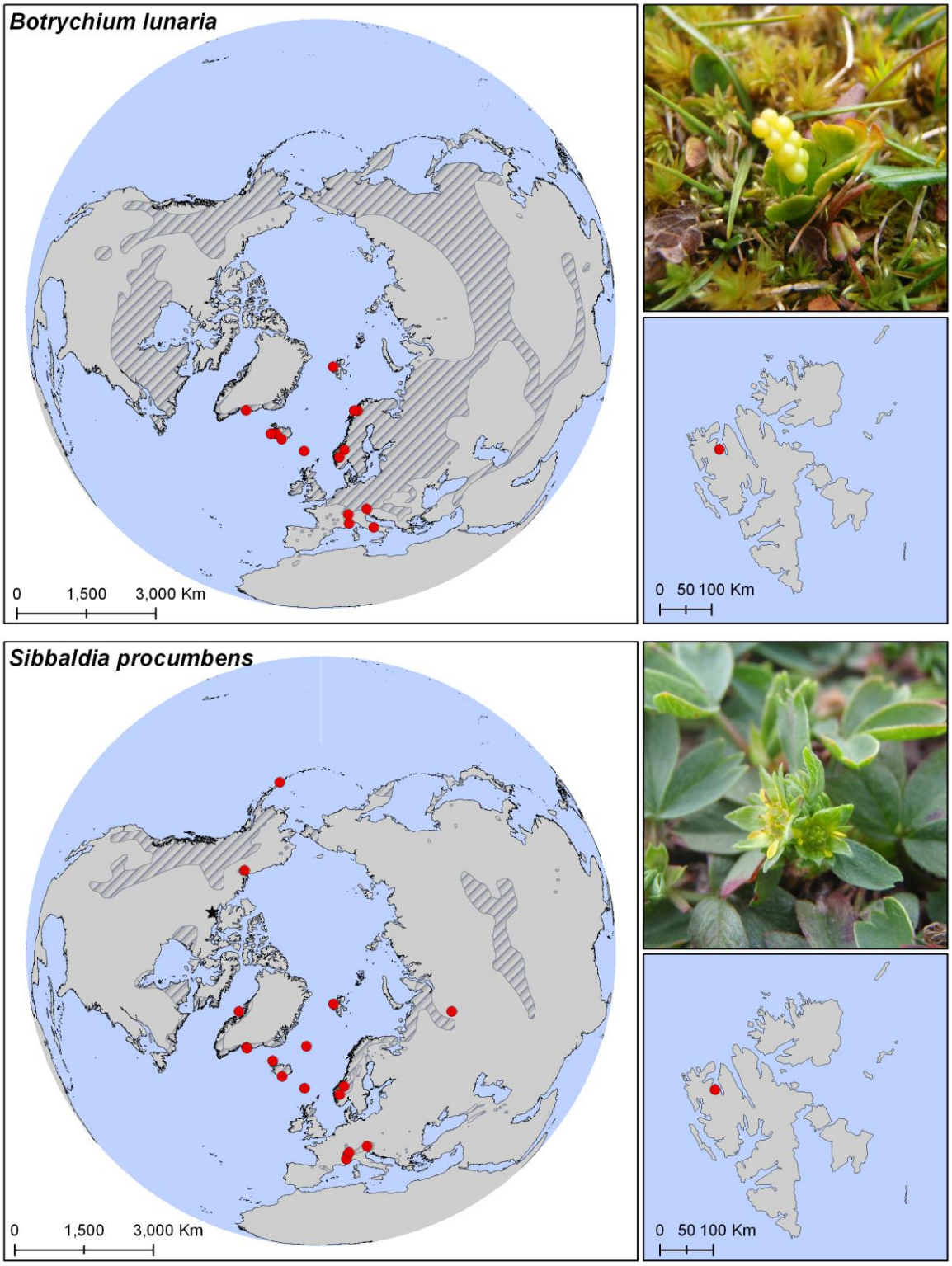


Figure 2. *Sampling map for Botrychium lunaria (top) and Sibbaldia procumbens (bottom). Distribution is indicated in stripes. Star = only one individual.*

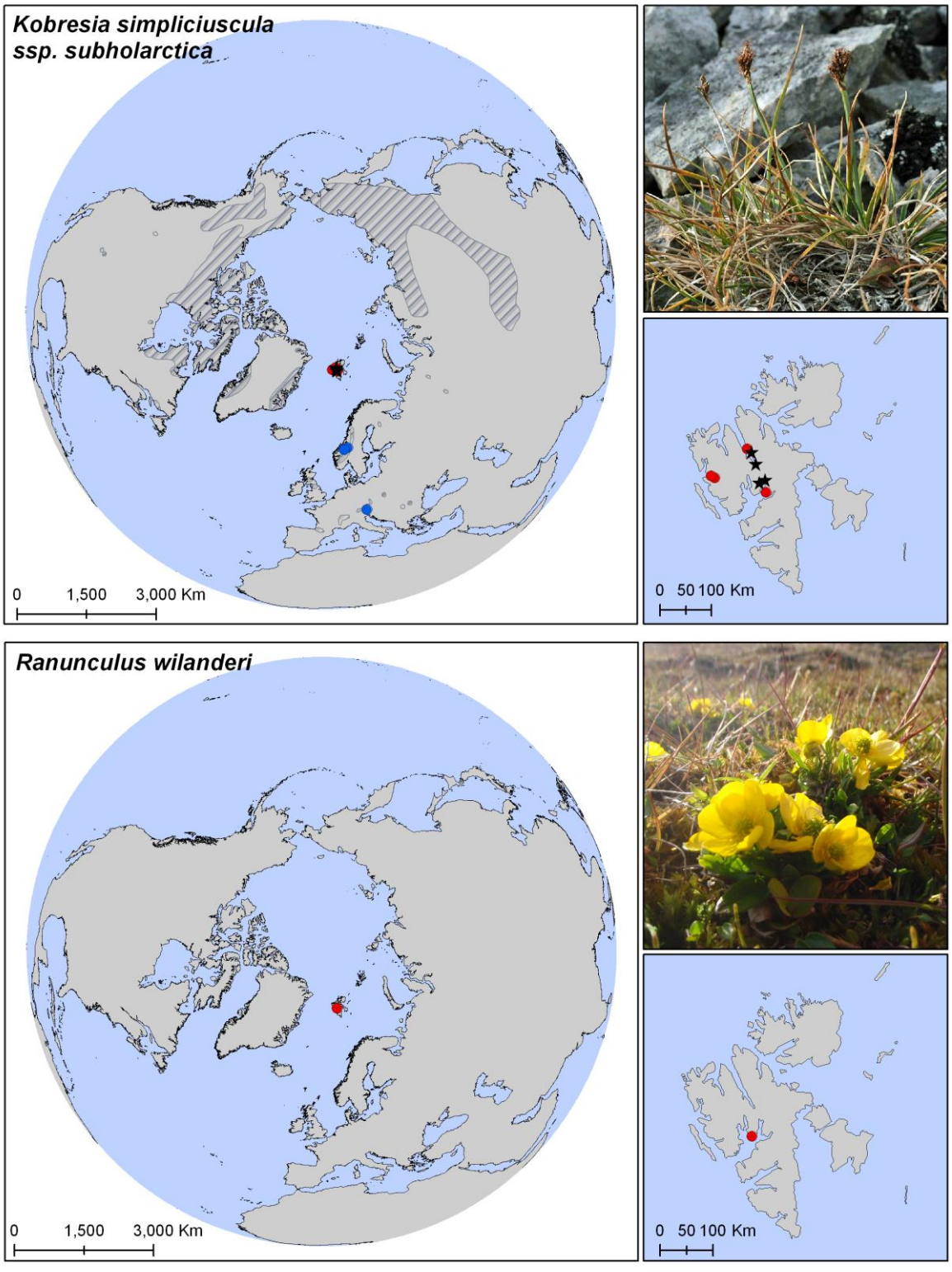


Figure 3. *Sampling map for Kobresia simpliciuscula ssp. subholarctica (top) and Ranunculus wilanderi (bottom).Blue dots = K. simpliciuscula ssp. simpliciuscula (the other subspecies), Stars = Svalbard locations, that was not sampled for genetic analyses.*

2.4.2 DNA extraction

For DNA extractions of *B. lunaria*, *K. simpliciuscula* ssp.*subholarctica* and *S. procumbens* two different methods (the E.Z.N.A.™ SP Plant DNA Mini Kit, Omega Bio-Tek, Norcross; and the Ziegenhagen protocol, see below) were tested for each species, before extraction of all samples. The final DNA extraction of *B. lunaria*, *S. procumbens* and *K. simpliciuscula* ssp. *subholarctica* followed the protocol of Ziegenhagen et al. (1993) after the procedure of Guillemaut and Maréchal-Drouard (1992), with the following modifications: Approximately 20 mg of leaves were crushed in 2 ml tubes with two tungsten carbon beads at 20 hz for 2 x 1 minute on a mixer mill (MM03, Retsch GmbH & Co, Haan). The samples were quickly spun down before a preheated (65° C) extraction buffer was added. The first centrifugation step was increased to 15 minutes at 13000 rpm, the second centrifugation step was increased to 20 minutes at 13000 rpm and the last centrifugation step was increased to 15 minutes at 13000 rpm. In addition, an extra purification step was added after the last centrifugation: 1 ml ice-cold 70 % ethanol was added to each sample, centrifuged for 2 minutes at 13 000 rpm, and then removed. This step was repeated before the samples were left over night to dry. The final DNA pellet was dissolved in 100 µl TE-buffer and 1 µl RNAse was added before the incubation at 37°C. The *R. wilanderi* samples were extracted in the laboratories of the University of Tromsø using the DNeasy™ Plant Mini Kit (Qiagen, Düsseldorf) following the manufacturer's protocol. For all extractions, 1-2 negative controls and 1-2 positive controls were included to ensure that possible contamination was discovered and that DNA product was reliable. DNA concentrations were measured with a NanoDrop spectrophotometer ND-1000 (Thermo Scientific, Wilmington), and ranged from just below 100 ng/µl to over 1000 ng/µl. Most samples had concentrations around 500 ng/µl. To make sure that all samples had approximately the same concentration prior to the AFLP procedure, they were diluted five (< 250 ng/µl), ten (250-749.9 ng/µl), twenty (750-1250 ng/µl) or thirty (> 1250 ng/µl) times. The DNA concentrations of *R. wilanderi* ranged from 13 to 17 ng/µl, and were kept undiluted.

2.4.3 AFLP analyses

To examine the genetic diversity we used the Amplified Fragment Length Polymorphisms (AFLP) multilocus genetic fingerprinting technique. The AFLP procedure followed the modifications of Jørgensen et al. (2006) on the original AFLP protocol by Vos et al. (1995) with a few exceptions: Instead of using 5.5 µl of isolated DNA per sample in the restriction-ligation step, only 2 µl were added. This gave a total restriction-ligation reaction volume of 11

µl. In the two amplification steps only 0.075 µl of AmpliTaq (Applied Biosystems, Foster City) and AmpliTaq Gold (Applied Biosystems, Foster City) was added to the pre-selective reaction mix and selective reaction mix respectively. The elongation step was extended to 2 min at 72°C in the pre-selective PCR-program, and to 1 min at 72°C in the selective PCR-program. All the incubations and amplifications were carried out on an Eppendorf Master cycler™ (Eppendorf, Hamburg). To ensure that the final fingerprints (or profiles), contained a reasonable amount of restriction fragments that were well spaced out, and also an appropriate amount of polymorphisms, a series of primer tests for the final selective amplification step were performed prior to the final run. We tested from 18 (*S. procumbens*) to 48 primer pairs (*B. lunaria*) per species on a selection of samples from different geographic regions. From these tests, four primer pairs were chosen for each species (Table 2). A set of negatives, replicates and duplicates was included in all final AFLP-runs to check for contamination and replicability (Bonin et al. 2004). Here replicates are considered to be the same sample included twice in the set up, and duplicates to be the same individual isolated twice. If an AFLP-run included several plates, replicates between the plates were also included. The final number of negatives, replicates and duplicates (Table 2) was a trade-off between available space per plate and a good representation of the sample set. The analysis of fragment lengths was performed on an ABI 3730 DNA analyzer (Applied Biosystems, Foster City). When primer combinations were labeled with different fluorescent colors, the selective reactions were co-loaded on the ABI sequencer. For each sample, 2 µl from a mix of co-loaded selective products (3 µl FAM, 3 µl NED, 3 µl PET and 2 µl VIC) were mixed with 0.3 µl GeneScan™ 500 (-250) LIZ size standard (Applied Biosystems, Foster City), and 11.7 Hi-Di™ formamide (Applied Biosystems, Foster City). Selective products of *S. procumbens* were run with only 8.85 µl HiDi formamide and 0.15 µl Liz size standard. The plate was vortexed, spun down and denatured at 95 ºC for five minutes. After denaturation, the plate was immediately put on ice for a few minutes and then run on the ABI sequencer. All of the DNA extractions (except for *R. wilanderi*) and AFLP analyses were performed in the CEES laboratory at the University of Oslo.

2.4.4 AFLP scoring

The AFLP electropherograms were analysed in GeneMapper version 3.7 (Applied Biosystems, Foster City), with a base pair range of 50-500 bp. The result is a profile where presence of a restriction fragment is represented by a peak at the appropriate base pair length. Making these profiles into a binary presence-absence table (scoring) has been shown to be the most error-prone step in the AFLP procedure, because it relies on subjective decisions (Bonin et al. 2004). A semi-automated method (AFLPScore) for scoring profiles in the R CRAN freeware was used for *S. procumbens*, *R. wilanderi* and *K. simpliciuscula* ssp. *subholarctica*. The method uses thresholds of AFLP-polymerase chain reaction product florescence intensity (peak height) in order to: (i) exclude AFLP loci that are likely to contribute to high error rates and (ii) determine the AFLP phenotype (fragment presence or absence) at the retained loci (Whitlock et al. 2008). An analysis of error rate is an integrated part of this procedure. AFLPScore contains a filtering option for removing loci that contains a lot of noise-peaks, which was applied for all three species. The final threshold (type and value) used for each primer combination was a trade-off between a high number of loci and a low error rate. The final binary table was checked by hand as recommended by Bonin et al. (2004). The *B. lunaria* profiles were scored manually. Although replicates were relatively similar, differences in peak morphology and intensity made it hard to use AFLPScore for this species. Altogether, 48 primer pairs were tested for this species and the AFLP procedure was tested with DNA extracted by two different protocols. Still finding primer combinations that gave readible profiles was overall difficult, which might be caused by PCR-inhibitors or the large genome (2n=90). Anyway, a satisfactory scoring was achieved by hand when loci with a high number of errors between replicates were removed. The mismatch error rate was calculated for each data set.

Table 2. *AFLP details. Total number of samples is given to the left, followed by the number of primer tests and the number of negatives (Neg.), replicates (Repl.) (replicates between plates in parentheses) and duplicates per AFLP run. EcoRI + MseI gives the primercombinations. (The EcoRI fluorescene color is given). Finally, there is the amount of genetic markers and the mismatch error rate.*

Species	Samples [n]	Primer tests [n]	Neg. [n]	Repl. [pairs]	Dupl. [pairs]	EcoRI	MseI	Markers [n]	Error rate [%]
B. lunaria [+ Outgroup]	111 [+5]	48	2	25 [8]	3	AAG **FAM** AGA **PET** ACG **VIC** ACA **NED**	CGA CGA CAC CTA	78	2.0
S. procumbens [+ Outgroup]	150 [+2]	18	2	14 [3]	6	AGG **VIC** ACA **NED** ACC **FAM** ACA **NED**	CAA CAT CAG CAC	145	1.2
K. simpliciuscula ssp. subholarctica [+ Outgroup]	54 [+5]	36	4	12	1	ACC **FAM** ACA **NED** AAG **VIC** AGA **PET**	CAG CTC CGA CGA	111	0.2
R. wilanderi [+ Outgroup]	19 [+5}	36	4	16	4	AAG **VIC** AGC **FAM** AGA **PET** ACG **VIC**	CGA CGG CAC CGA	169	0.9

2.4.5 Statistical analyses of AFLP-data

For each of the four species, Nei's gene diversity (D) was calculated as the average proportion of pairwise differences between individuals in a population (Kosman & Leonard 2005). In addition, the percentage of polymorphic loci was calculated as comparable measure. The number of genotypes, or clones, in each population was also estimated according to Nei's formula for haplotype diversity (Nei 1987). In order to investigate if the Svalbard populations might be divergent genetic lines with many rare markers, The Frequency-Down-Weighted marker values (DW) were calculated according to Schönswetter and Tribsch (2005). The number of occurrences of each marker in a certain population was divided by the total number of occurrences of that marker in the total data set. Normally these marker values are summed up to the DW-value for each population, but because of the uneven sample size the ratio of means was used according to Ehrich (2006). The calculations of D, the percentage of polymorphic loci, DW and the number of genotypes were performed with AFLPdat (Ehrich 2006) in the R CRAN freeware version 2.13.0. To investigate genetic structure and establish

the Svalbard populations' relationship to the rest of the species, principal coordinates analyses (PCoA) plots were made in Past version 2.13 (Hammer et al. 2001) and neighbor-nets were constructed in SplitsTree version 4.12.3 (Huson & Bryant 2006). Both the Hamming distance measure and the Jaccard distance measure were used when constructing PCoA plots and Neighbor-nets. Further analyses were only performed on the *S. procumbens* and *B. lunaria* data sets, because of the limited amount of data for *R. wilanderi* and *K. simpliciuscula* ssp. *subholarctica*. Possible genetic groups were delineated using a Bayesian approach as implemented in Structure version 2.3 (Pritchard et al. 2000). The model accounts for the presence of Hardy-Weinberg or linkage disequilibrium by introducing population structure and attempts to find population groupings that (as far as possible) are not in disequilibrium (Pritchard et al. 2000). We used the recessive allele model for dominant markers with assumed admixture and with uncorrelated allele frequencies. The number of possible groups, K, ranged from one to the total number of sampling localities for each species (1-18 for *S. procumbens* and 1-15 for *B. lunaria*). Ten independent runs were carried out for each number of K with a burn-in period of 10^5 and 10^6 Markov Chain Monte Carlo replicates after burn-in. The Structure program was run at Bioportalen at the University of Oslo (https://www.bioportal.uio.no/). Optimal clustering of individuals was inferred based on the mean logarithmic likelihood of K values, similarity coefficients for all runs for each K and delta K, all calculated with Structure-sum version 2011 (Ehrich 2006). To test for significant divergence among groups, analyses of Molecular Variance (AMOVA) were carried out in Arlequin version 3.5 (Excoffier et al. 2005). Components of variance partitioned within populations, among populations and among potential genetic groups as detected in the PCoA plots, Neighbor-nets and Structure analyses, were estimated from a distance matrix based of the number of pairwise differences. F-statistics were replaced by the analogous Φ-statistics, as recommended for binary data by Excoffier et al. (1992). Finally to find the possible nearest relative of the Svalbard populations, we performed an allocation test with AFLPOP version 1.1 (Duchesne & Bernatchez 2002). The program allocates individuals to populations based on the log likelihoods of the multilocus genetic data. The default is to allocate an individual to a population as soon as the likelihood of one population is higher than all others, but the threshold for allocation can be set by the user. We used the highest log-likelihood difference where allocation still occurred, which was an allocation threshold of 1 for *B. lunaria* and a threshold of 2 for *S. procumbens*. This means that an individual was only allocated to a certain population, if assignment to this population was at least 10 times more likely than for all other

populations in the case of *B. lunaria*, and a 100 times more likely in the case of *S. procumbens*. Marker frequencies of zero were replaced with 0.001.

3. Results

3.1 Localities, population sizes, potential threats and implications for red list categories

During our visit to Bockfjorden in August 2009, we found 21 shoots of *B. lunaria* growing on a small green patch (33 m^2) right beside the warmest springs in the Troll spring complex. We measured the temperature of this spring to be 23°C at the surface. All the 21 *B. lunaria* shoots were not higher than 3-4 cm. The spot was heavily grazed and might also be vulnerable to trampling from visiting tourists. According to Elvebakk and Spjelkavik (1981) the springs sometimes dry out or shift location. Since *B. lunaria* is growing in such a close proximity to a hot spring, this might be another potential threat for the population. The species was already in the highest red list category Critically Endangered, (CR), and our information did not change that (Table 3).

Sibbaldia procumbens was growing in the slopes just 0.5 to 20 meter away from the springs. The species seemed to especially thrive in small depressions in the slopes. The distribution area was continuous, covering approximately 3000 m^2 (600 m × 5 m). Population size was estimated to be over a thousand individuals, and one quarter of them was flowering. The habitat was typically dry to medium dry snowbeds. As in the *B. lunaria* patch, the area was heavily grazed, but the grazing diminished higher up in the slopes. When it comes to human disturbance, trampling is probably not a problem in the steeper part of the slopes and in the depressions, as it may be in the areas closer to the springs. The substrate in the slopes seemed to be quite stable. Due to larger population size than previously thought and several flowering individuals, *S. procumbens* was moved up from the Critically Endangered category (CR) to the "Endangered" category (EN) (Table 3).

Twenty tussocks of *Kobresia simpliciuscula* ssp. *subholarctica* were found in Gipsvika, growing in an alkaline mire with a lot of standing water. Geese and reindeer grazing were detected in close proximity, but not on the *K. simpliciuscula* ssp. *subholarctica* tussocks. Because of the wet location, trampling from tourists is probably not a problem. The two *K. simpliciuscula* ssp. *subholarctica* locations in the Kongsfjorden area in Haakon VII Land, were located in short distance from each other. The population at Blomstrandøya consisted of 14 tussocks within 200 m^2, while the population at Ossian Sarsfjellet consisted of 60 tussocks

distributed over 800 m^2. The population at Ossian Sarsfjellet is probably the largest population of *K. simpliciuscula* ssp. *subholarctica* in Svalbard. The species was growing in a sheltered warm slope in a moist mire. Some grazing was registered in addition to a hiking track that was situated not far from the population. In the Blomstrandøya population, plants were growing in a more gravelly and dry substrate than at Gipsvika and Ossian Sarsfjellet. Three of the tussocks were under 12 cm in diameter, but the rest were over 12 cm. No grazing or sign of human disturbance was detected. In Wijdefjorden nine tussocks (one growing a bit further away from the others) were found within 75 m^2 in Flatøyrdalen. No signs of grazing or human disturbance were registered. At the other Wijdefjorden locality, Lemströmfjelllet, 1-3 tussocks of *K. simpliciuscula* ssp. *subholarctica* were found within 1 m^2 in early July 2011. It is not sure if these tussocks belonged to the population of 50 individuals reported by Elvebakk and Nilsen (2002). No threats were observed. We did not manage to visit Reinsbukkdalen, Adolfbukta or Mimerdalen during the time period for this study. *Kobresia simpliciuscula* ssp. *subholarctica* was kept in the red list category Endangered (EN) in the regional red list of 2010 (Table 3).

At the *R. wilanderi* locality, we counted 51 individuals in a green, moist area of 2000 m^2. There were no apparent threats in the area (erosion, human disturbance etc.). Moderate grazing levels was detected, but normally members of the *Ranunculaceae*-family produce poisonous substances that make herbivores avoid them (Elven et al. 2007). *Ranunculus wilanderi* was moved from the previous Critically Endangered category (CR) to the Endangered category (EN) on the basis of the population size (Table 3).

Table 3. *Population size estimations in the different localities. Some of the* Kobresia simpliciuscula *ssp.* subholarctica *localities, Mimerdalen, Reinsbukkdalen and Adolfbukta, were not visited in this study and are therefore not shown. (See discussion).*

Species	Locality	Number of individuals	Distribution area (m^2)	Total number of individuals,Svalbard	Red list category (2006)	Red list category (2010)
Botrychium lunaria	Bockfjorden (Haakon VII Land)	21	33	**21**	**CR**	**CR**
Sibbaldia procumbens	Bockfjorden (Haakon VII Land)	>1000	3000	**>1000**	**CR**	**EN**
Kobresia simpliciuscula ssp. *subholarctica*	Gipsvika (Bünsow Land)	20*	246	**124-146***	**EN**	**EN**
	Ossian Sarsfjellet (Haakon VII Land)	60*	800			
	Blomstrandøya (Haakon VII Land)	14*	200			
	Flatøyrdalen (Ny Friesland)	9*	75			
	Lemnöfjellet (Ny Friesland)	1-3*	1			
Ranunculus wilanderi	Kapp Thordsen (Dickson land)	51	2000	**51**	**CR**	**EN**

3.2 Ecological investigations

3.2.1 Summary statistics of the explanatory variables

The habitat of *B. lunaria* in Bockfjorden was a bright green patch with a lot of bryophytes (mean=65 %, n=4; Table 4) and vascular vegetation cover (mean=40 %, n=4). The moisture levels ranged from 2-3, but lay generally closer to a 3 ("wringing wet"; mean=2.75, n=4). The temperature measured in the *B. lunaria* habitat was the highest measured in the Bockfjorden area, probably because of the proximity to the warmest spring. The mean of the four pH measurements was 6.7, ranging from 6.4 to 7.2. The cover of cryptogamic crust was around 15 %, but ranged from 0 % to 60 %.

Sibbaldia procumbens was found in the slopes above the Troll springs where bryophyte cover was generally less than in the *B. lunaria* patch (mean=35.6, n=9), but the vascular vegetation cover higher (mean= 70 %, n=9). The moisture level was overall between "dry" and "moist" (mean=1.44, n=9). Some lichen and cryptogamic crust could be found in the plots (mean=0.3 % and 7.2 % respectively). The highest pH value recorded among all the focus species plots, was measured in the *S. procumbens* plots and reached as high as 7.7. But the mean of the pH values was the same as for *B. lunaria*, 6.7 (n=9). The slope in the *S. procumbens* habitat was the steepest of all the focus species habitats (mean=22.1°, min=5°, max=50°, n=9).

The *K. simpliciuscula* ssp. *subholarctica* mires had the highest pH values in general, with an overall mean of 7.3 (n=11). The plots from Gipsvika had mean pH value of 7.1 (n=6), and the plots from Ossian Sarsfjellet a mean value of 7.5 (n=5). The moisture regime varied from 2-4, ("moist" to "dripping wet"), with Gipsvika being the wettest locality with a mean of 3.17 (n=6) and a lot of standing water. Cover of cryptogamic crust in the *K. simpliciuscula* ssp. *subholarctica* plots was the highest measured for all the focus species, with a mean of 23.0 % (n=11) for all sites. In Gipsvika, cover of cryptogamic crust reached as high as 90 % (Gipsvika, mean=35.8, n=6). The vascular vegetation cover was quite high in both sites (mean=53.2, n=11) - probably partially an artifact of the tussock-habit of the focus species itself. The plots also inhabited quite an extensive bryophyte cover, with a mean of 28.1 % (n=11). The *K. simpliciuscula* ssp. *subholarctica* habitat had the most bare ground and stones of all the focus species (stones and bare ground together, mean=6.7 %, n=11).

Ranunculus wilanderi was growing in a green moist area (mean=3.2, n=5) with a lot of bryophytes (mean=83.0 %, n=5) and vascular cover (mean=47.4 %, n=5). The bryophyte levels were the highest measured among the focus species, reaching as high as 100 % cover (n=5). Lichen and cryptogamic crust cover lay around 1.2 % and 4.0 % respectively (n=5), while the mean pH value was 6.5.

Values are summarized in Table 4.

3.2.2 Wilcoxon rank sum test

Significant differences ($p<0.05$) between focus species plots and control plots were just found for *S. procumbens* and both sites of *K. simpliciuscula* ssp. *subholarctica* together. The *S. procumbens* focus species plots had a lower moisture level (p=0.0477) and a higher temperature at both 3 cm (p = 0.0039) and 10 cm (p=0.0078) than the control plots. Further, it was also a significant difference in aspect between the two plot types (p=0.0346), with the focus species plots facing a more south to southeast direction than the control plots. For the two *K. simpliciuscula* ssp. *subholarctica* sites analyzed together, the focus species plots had higher cover of bryophytes (p=0.0330) and vascular cover (p=0.0144) than the control plots. Opposite, the control plots had a higher cover of cryptogamic crust (p=0.0467) and bare ground and stones (p=0.0124) than the focus species plots.

Abbreviations in figures and tables for the ecological part:

pH = pH
Moist = Moisture level
Temp3 = Temperature at 3 cm
Temp10 = Temperature at 10 cm
Vasc = Vascular cover
Bryo = Bryophyte cover
Lichen = Lichen cover
Crypto = Cryptogamic crust cover
Stones = Cover of stones
Bare.gr. = Cover of bare ground
Slope = Slope

Table 4.*Mean and range of all explanatory variables. White rows indicate the focus species plots, while grey rows indicate the control plots.* = Cover of bare ground and stones estimated together. NA = missing values. For explanations of abbreviations see box on previous page.*

Species	pH	Moist [1-4]	Temp3 [°C]	Temp10 [°C]	Vasc [%]	Bryo [%]	Lichen [%]	Crypto [%]	Stones [%]	Bare.gr [%]	Slope [°]
B. lunaria	6.7	2.75	14.5	15.8	40.0	65.0	0.0	15.0	1.4	0.0	8.5
	[6.4-7.2]	[2-3]	[13.9-14.9]	[15.1-16.2]	[30.0-50.0]	[20.0-90.0]	[0.0-0.0]	[0.0-60.0]	[0.0-4.0]	[0.0-0.0]	[0.0-15.0]
	6.9	3.00	14.0	16.1	60.0	86.3	0.0	5.0	0.0	0.0	10.5
	[6.3-8.1]	[3-3]	[13.8- 14.2]	[15.7- 16.5]	[50.0-70.0]	[50.0-100.0]	[0.0-0.0]	[0.0-20.0]	[0.0-0.0]	[0.0-0.0]	[4.0- 16.0]
S. procumbens	6.7	1.44	11.2	11.9	70.0	35.6	0.3	7.2	1.6	0.4	22.1
	[6.1-7.7]	[1-2]	[9.3-14.9]	[9.5-15.4]	[30.0-100.0]	[0.5-90.0]	[0.0-2.0]	[0.0-20.0]	[0.0-10.0]	[0.0-2.0]	[5.0-50.0]
	7.0	2.10	9.9	10.6	58.3	40.1	0.1	14.6	0.1	0.0	13.3
	[6.5- 8.8]	[2-3]	[8.8- 12.6]	[9.4- 14.1]	[20.0- 95.0]	[1.0-75.0]	[0.0-0.5]	[0.0-50.0]	[0.0-1.0]	[0.0-0.0]	[4.0- 22.0]
K. simpliciuscula	7.3	2.73	10.3	NA	53.2	28.1	0.6	23.0	NA	6.7*	NA
ssp. *subholarctica*	[6.5-7.6]	[2-4]	[6.9-13.6]		[10.0-90.0]	[0-80]	[0.0-5.0]	[2.0-90.0]		[0-37.0]	
	7.3	3.10	10.6	NA	20.6	9.0	0.1	40.9	NA	28.5*	NA
	[6.8- 7.5]	[2-4]	[7.8- 14.2]		[2.0- 75.0]	[0.0-40.0]	[0.0-1.0]	[0.0-80.0]		[0.0-90.0]	
- Gipsvika	7.1	3.17	12.4	NA	63.3	25.5	0.0	35.8	NA	1.7*	NA
	[6.5-7.6]	[3-4]	[11.4- 13.6]		[10.0-90.0]	[0-80]	[0.0-0.0]	[5.0-90.0]		[0.0-5.0]	
	7.2	3.70	12.6	NA	18.3	4.0	0.0	58.3	NA	23.3	NA
	[6.8- 7.4]	[3-4]	[11.6-14.2]		[5.0- 35.0]	[0.0-15.0]	[0.0-0.0]	[10.0-80.0]		[0.0-50.0]	
- Ossian Sarsfjellet	7.5	2.20	7.9	7.7	41.0	31.2	1.4	7.5	5.0	7.8	8.2
	[7.3-7.6]	[2-3]	[6.9-8.4]	[7.5-8.2]	[25.0-80.0]	[6.0-70.0]	[0.0-5.0]	[2.0-12.0]	[1.0-13.0]	[0.0-24.0]	[4.0-12.0]
	7.4	2.40	8.1	7.8	23.2	15.0	0.3	20.0	21.8	12.8	10.2
	[7.1- 7.5]	[2-3]	[7.8-8.4]	[7.6- 8.1]	[2.0-75.0]	[2.0- 40.0]	[0.0-1.0]	[0.0-55.0]	[8.0-45.0]	[0.0-45.0]	[6.0- 18.0]
R. wilanderi	6.5	3.20	11.3	5.7	47.4	83.0	1.2	4.0	0.0	0.0	4.4
	[6.2-6.8]	[3-4]	[8.1-13.7]	[3.3-9.5]	[25.0-70.0]	[50.0-100.0]	[0.0-5.0]	[0.0-14.0]	[0.0-0.0]	[0.0-0.0]	[0.0-10.0]
	6.6	3.20	10.3	5.9	44.2	91.0	0.8	1.4	0.0	0.0	2.4
	[6.2- 6.9]	[2-4]	[8.0-12.4]	[5.1-7.9]	[25.0- 70.0]	[60.0-100.0]	[0.0-2.0]	[0.0-3.0]	[0.0-0.0]	[0.0-0.0]	[0.0-6.0]

3.2.3 Correlations between explanatory variables

Many explanatory variables were correlated, and the results were taken into consideration when interpreting the ecological results. However, due to the extent of the tables, they are given in the appendix.

3.2.4 Ordinations

In the ordination for the entire area around Trollkjeldane in Bockfjorden, (n=60), all three GNMDS axes (k=3) were correlated with at least one of the DCA axes (DCA1 and GNMDS1: τ=0.7682, p=<2.2e-16, DCA2 and GNMDS2: τ=0.5542, p=5.66E-10. DCA3 and GNMDS3: τ=0.4513, p=4.45E-07 and DCA4 and GNMDS3: τ=0.5593, p=3.89E-10). The result was therefore considered reliable, and the final DCA with overlaid explanatory variables is shown in Figure 4. High variation was indicated by the eigenvalues of DCA axes 1 (0.5571) and 2 (0.3755) and axis length of 4.15 S.D and 2.78 S.D. units, respectively. The *S. procumbens* plots (represented by blue and light blue control plots) grouped closed together at the left side of DCA axis 1 and the upper side of DCA axis 2, close to the origin. The *B. lunaria* plots also made a distinct group with approximately the same placement along DCA axis 1 as *S. procumbens*, but at the lower end of DCA axis 2. Among the explanatory variables, pH and bare ground cover were positively correlated, while vascular cover and bryophyte cover were negatively correlated with DCA axis 1 (values given in appendix). Both the *B. lunaria* plots and the *S. procumbens* plots seemed, thus, to be associated with higher vascular plant and bryophyte cover and lower pH levels than was otherwise registered in the other Bockfjorden-plots. Temperature at 10 cm, temperature at 3 cm and moisture level were all negatively correlated with DCA axis 2 (values given in the appendix), indicating an increase in temperature and moisture level towards the lower part of the ordination plot where the *B. lunaria* plots are found. As the arrows for each variable are pointing in the direction of maximum increase, the analysis might indicate that cryptogamic crust cover, slope and lichen cover increase towards the upper part of the ordination plot where the *S. procumbens* plots are located, but none of these variables were significantly correlated with DCA axis 2. Aspect seemed not to explain any of the gradients. Of the focus species from the other study, the *Carex capillaris* plots are closest to and somewhat scattered between *S. procumbens* and *B. lunaria*, whereas the *Tofieldia pusilla* plots are situated close to the *S. procumbens* plots at the upper end of the ordination plot. In the field, *C. capillaris* was

found in both the *B. lunaria* and *S. procumbens* plots (and vice versa), and *S. procumbens* was also found in the *B. lunaria* plots.

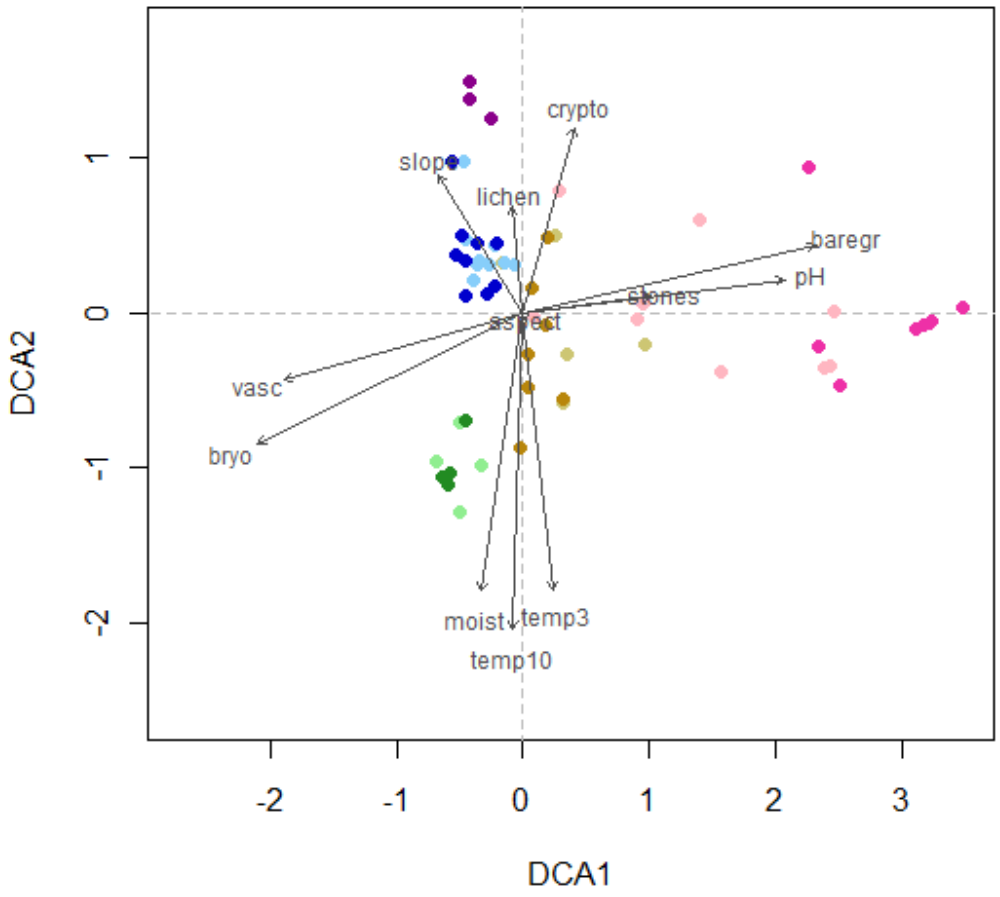


Figure 4. *DCA of the entire area around Trollkjeldane with overlayed explanatory variables. Green =* ***Botrychium lunaria****, Blue =* ***Sibbaldia procumbens****, Brown =* ***Carex capillaris****, Pink =* ***Puccinellia svalbardensis****, Purple =* ***Tofieldia pusilla****.* ***Controlplots*** *are indicated by a* ***lighter color****. For explanatory variables see earlier abbreviation box.*

In the ordinations done for each of the Bockfjorden species separately, the *B. lunaria* ordination (not shown) was based only on eight plots, which made it hard to interpret the results. Although τ for all three axes of GNMDS (k=3) was correlated with one of the four DCA axes, none of the p-values for these correlations showed significance below p=0.05, but from the ordination plot the eight plots looked intermingled.

The ordination of *S. procumbens* was based on 18 plots. The two GNMDS axes (k=2) were both correlated with one of the four DCA axes (GNMDS1 and DCA1: $\tau = 0.6667$, p=0.0001, GNMDS2 and DCA3: $\tau = 0.4500$, p = 0.0152). The eigenvalues of DCA axes 1 and 2 were 0.2020 and 0.1350 respectively and the axis length 1.54 S.D. units for DCA axis 1 and 1.51

S.D. units for DCA axis 2. The plots with the focus species and the control plots seemed to be distributed quite evenly along DCA axis 1, but there was a slight tendency for separation between the two plot types along DCA axis 2. The only correlation between the explanatory variables and the two first DCA axes was a positive correlation between temperature at 10 cm and 3 cm and DCA axis 2 (values given in appendix) which might indicate a slightly warmer temperature in the focus species plots compared to the control plots (similar to the results from the Wilcoxon rank sum test). However, when removing *S. procumbens* from the species matrix, the two plot types were a lot more mixed in the resulting ordination plot (Figure 5), indicating that the species composition in the two plot types were generally the same. The removal of *S. procumbens* from the species matrix, also removed the correlation between temperature and DCA axis 2.

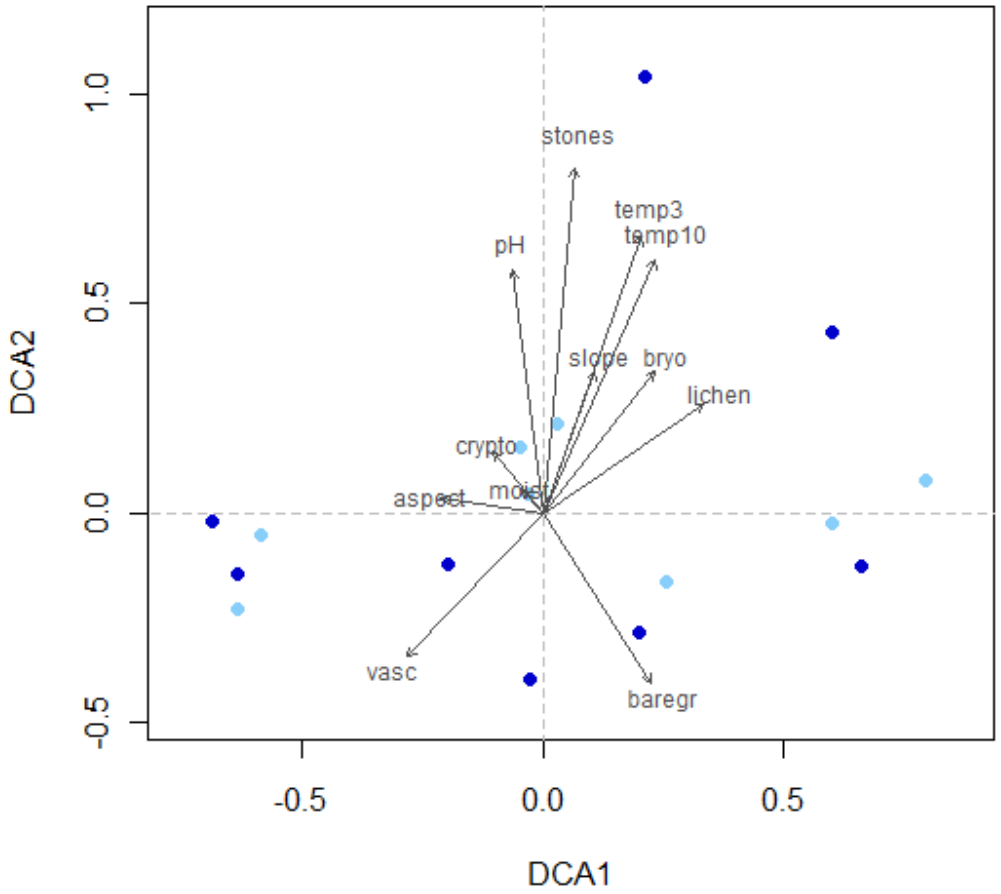


Figure 5. *DCA of the habitat of* ***Sibbaldia procumbens*** *with overlaid explanatory variables –* ***The focus species is removed from the input matrix****. Dark blue dots =* ***the focus species plots****, Light blue dots =* ***control plots.*** *For explanatory variables see earlier abbreviation box.*

Ordinations were performed separately for each of the *K. simpliciuscula* ssp. *subholarctica* sites, Gipsvika, (n=12) and Ossian Sarsfjellet (n=10), and also for both sites together (n=22). In the latter, both GNMDS axes (k=2) were correlated with one or more of the four DCA axes (GNMDS1 and DCA1: τ = -0.7143, p = 3.07E-07, GNMDS2 and DCA2 : τ = 0.4978, p = 0.0009, GNMDS2 and DCA3: τ = 0.5671, p = 0.0001, GNMDS2 and DCA4: τ = 0.5671, p = 0.0001), and the DCA was therefore considered reliable. Eigenvalues of DCA axes 1 and 2 were 0.3550 and 0.2732 respectively, and the axis length 3.06 S.D. units for DCA axis 1 and 1.98 S.D. units for DCA axis 2. So compared with the DCA results for *S. procumbens*, a lot more variation were explained by the first two axes (Figure 6). The plots from the two sites are intermingled in the ordination plot, implying that species composition must be quite similar. Along DCA axis 1, which explains most of the variation, both control plots and plots with the focus species are distributed quite evenly. However, along DCA axis 2 there seems to be a tendency that most control plots, except G1b, are located in the lower part of the ordination plot, whereas the plots with the focus species are generally located in the upper part (except O1a and G6b). When the explanatory variables were overlaid on the DCA (not shown), only one significant correlation was found between the axes and the variables. The amount of stones and bare ground (here estimated together), were significantly negatively correlated with DCA axis 2 (τ = -0.4436, p = 0.0046). Because *K. simpliciuscula* ssp. *subholarctica* has a tussock habit that normally occupies most of a plot, it makes sense that more bare ground is found in the control plots. When removing *K. simpliciuscula* ssp. *subholarctica* from the species matrix, the two plot types were a lot more mixed (not shown), indicating that the presence of the focus species itself was the main difference between the plot types.

The same accounted for the two plot types when just focusing on *K. simpliciuscula* ssp. *subholarctica* in Gipsvika. No ecological differences were detected between the two plot groups before the focus species was removed from the matrix. The GNMDS confirmed the DCA, with both GNMDS-axes (k=2) being correlated with one of the DCA-axes (GNMDS1 and DCA1: τ = 1, p = 4.18E-09, GNMDS2 and DCA2: τ = -0.4848, p = 0.0311). Eigenvalues were 0.5257 for DCA axis 1 and 0.1386 for DCA axis 2, and axis lengths 3.09 and 1.30 respectively. The DCA for the Ossian Sarsfjellet plots failed to be reproduced in the GNMDS. A significant correlation was found between the first GNMDS axis and DCA axis 1 (τ = -0.7333, p = 0.0022), but not for GNMDS axis 2 and any other DCA axes, with k = 2. Increasing the number of dimensions in the GNMDS did not seem to solve the problem. The

reason why there was not full reproducibility in the GNMDS is not known, but it might be a result of low sample size and low variation in the data set. The GNMDS (not shown) separated the plots with the focus species and the control plots, but the DCA did not.

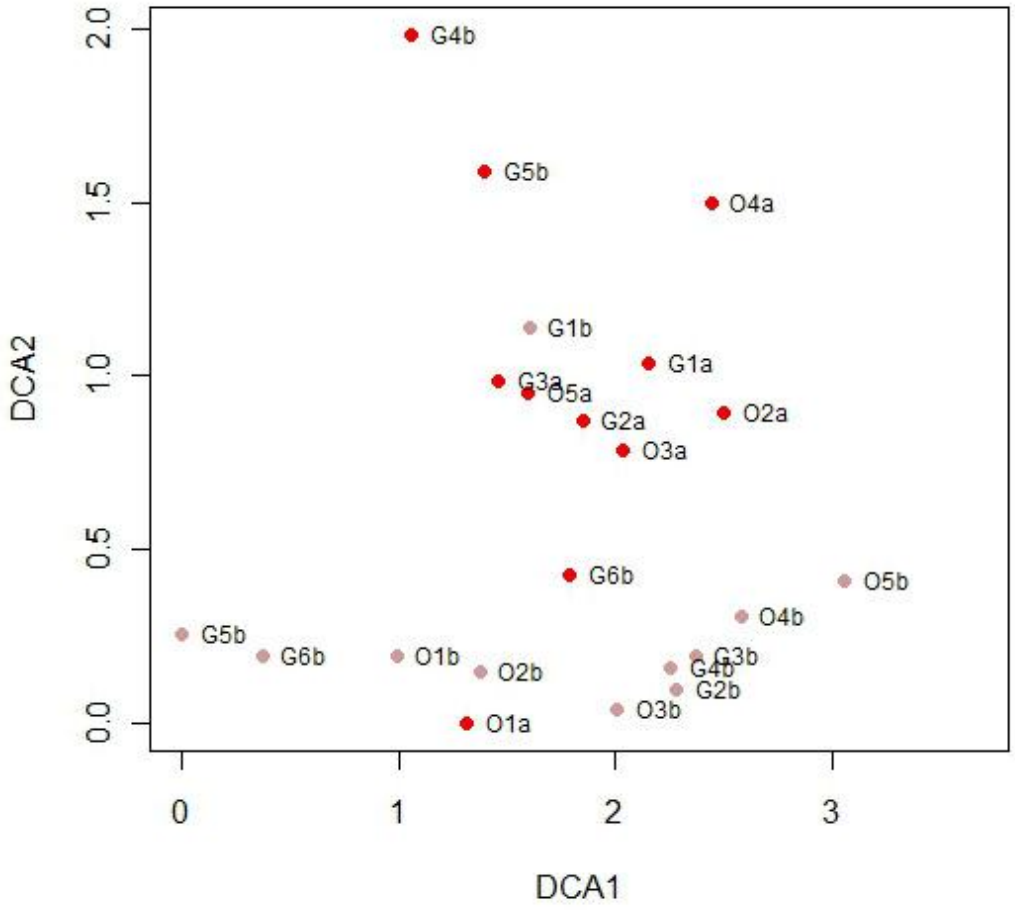


Figure 6. *DCA with plots from both* ***Ossian Sarsfjellet*** *and* ***Gipsvika****,* ***without explanatory variables****. Red =* ***Focus species plots****, Light red =* ***Control plots****. Plot names starting with* ***G = Gipsvika****, while plot names starting with* ***O = OssianSarsfjellet****.* ***The plot pairs are indicated with 1a and 1b, 2a + 2b etc.***

The DCA done for the *R. wilanderi* plots was confirmed by the GNMDS. Both GNMDS axes (k=2) were correlated with one or more of the DCA axes (GNMDS1 and DCA1: $\tau = 0.8667$, $p = 0.0001$, GNMDS2 and DCA2: $\tau = 0.8222$, $p = 0.0004$ and GNMDS2 and DCA4: $\tau = 0.6444$, $p = 0.0091$). In the DCA plot (not shown), the two plot types seemed to be intermingled and no useful information could be withdrawn from the explanatory variables, indicating that species composition was generally the same in the two plot types.

3.3 Genetic investigations

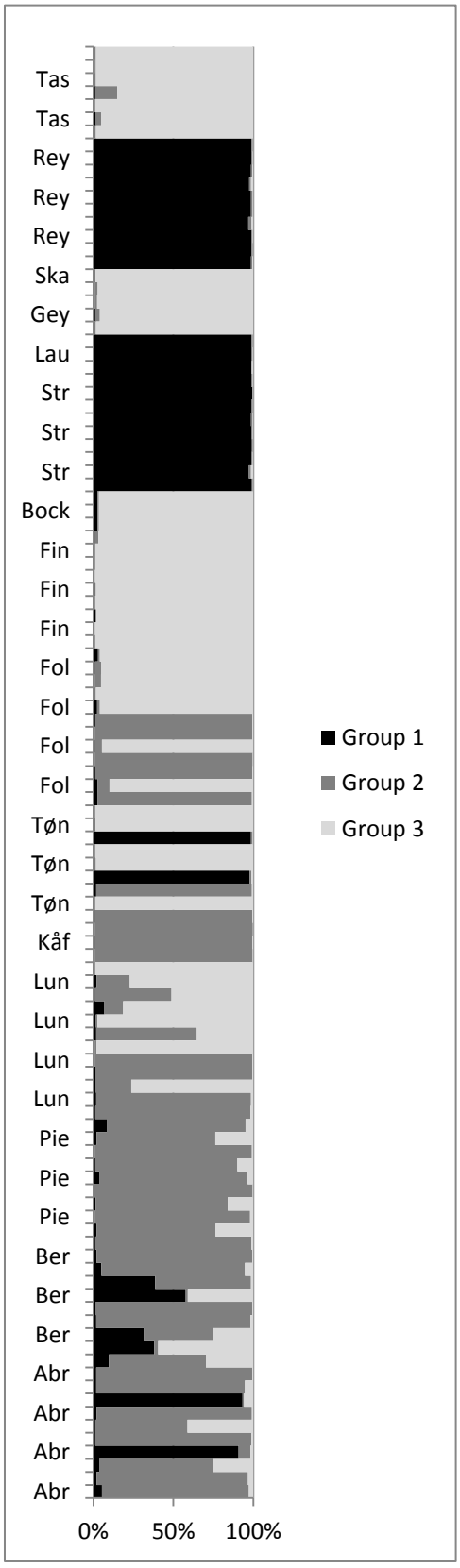
Final error rate, total number of loci and can be viewed in Table 2.

3.3.1 *Botrychium lunaria* – genetic results

The *B. lunaria* population in Bockfjorden showed no genetic diversity and only one genotype, and had the lowest D value of all populations (D = 0.000). The mean D value for all *B. lunaria* populations was 0.063, ranging from 0.000 (Bockfjorden) to 0.102 (Lungau in Austria, n=10). Among the reference populations, the distribution of genetic diversity followed a nearly geographic pattern. The populations from southern Europe had highest D values, followed by the populations from mainland Norway and the Geysir population from Iceland. These were all over the mean D value. Under the mean D value followed The Faroe Islands, Iceland and Greenland. (When looking at the percentage of polymorphic loci, the order was even more complete: Southern Europe – mainland Norway, The Faroe Islands - Greenland – Iceland – Svalbard). (Table 1, Figure 12).

As to the DW-values, *B. lunaria* from Bockfjorden had a ratio of means of 91.8 (n=3, Table 1). This is nearly the exact same value as the average for all *B. lunaria* populations (mean =91.7). The geographic pattern observed in the distribution of genetic diversity was somewhat less clear for the DW values, but generally all southern Europe populations had high DW values, mainland Norway moderate DW values and Greenland and most Iceland populations low DW values. The largest exception to this pattern was probably Geysir in Iceland with the second highest DW value. (Table 1, Figure 12).

There were no considerable differences between the PCoA plots constructed using the Jaccard distance measure and the Hamming distance measure. Therefore, only plots based on the Hamming distances are discussed here and for the other species. The PCoA plot of *B. lunaria* is shown in Figure 8. The first axis represented 30.4 % of the variation, the second 11.9 % and the third axis 5.1 %. The third axis gave no additional information to the first to axes. No very definitive groups were apparent in the PCoA plot, but there were tendencies for geographic structure. For instance along the first axis, the southern Europe populations seemed to group in the middle of all the *B. lunaria* individuals and there was also a small cluster of populations from Iceland and The Faroe Islands located to the right. On the other hand, many individuals from the mainland Norway populations seemed to appear here and there (except the Finse population) and two closely situated localities from Iceland, Geysir and Laugarvatn, appeared on different sides of the *B. lunaria* cluster along the first axis. The



three individuals from Bockfjorden were located on the left together with several individuals from the mainland Norway populations, Tasiilaq in Greenland and Skaftafell and Geysir in Iceland. Although *Botrychium boreale* was separated from *B. lunaria* along the first axis, the distance was relatively short between the two species. The neighbor-net showed overall the same tendencies as in the PCoA plot (not shown). The larger splits of the network 1) separated the group of individuals from the Faroe Islands and Iceland (also indicated in the PCoA plot) and 2) separated individuals at the upper part of PCoA plot (including most Central European individuals and some from mainland Norway, Greenland and Iceland) from the individuals at the lower part of the PCoA plot (including most individuals from mainland Norway and Greenland as well as some from Central Europe and Iceland in addition to the three Bockfjorden individuals). The three individuals from Bockfjorden were, in the neighbor-net closest to individuals from Skaftafell in Iceland. Genetic structure was also inferred with a Bayesian approach implemented in the software Structure. The optimal number of clusters was inferred to be three (K = 3) (Figure 7). The clusters were the same as the PCoA plot and the neighbor-net (somehow clearer) showed tendencies for: 1) Mainly individuals from Reykjanes and Laugarvatn in Iceland and Strendur in The Faroe Islands, but also a few individuals from Tønsvikdalen in mainland Norway and Abruzzo and Bern in southern Europe,

Figure 7 *Summary plot of three clusters within Botrychium lunaria inferred with the Structure software. Each individual is represented by a bar, which is divided into K segments. The length of each segment corresponds to each individual's mean logarithmic likelihood for each K. Each individual is represented by the three first letters of its population name.*

2) The Bockfjorden population, the Tasiilaq population from Greenland, The Geysir and Skaftafell population from Iceland, a few individuals from Lungau and Bern in southern Europe and most of the mainland Norway individuals 3) Most of the southern Europe individuals and a few individuals from mainland Norway.

The southern Europe populations showed most tendencies for division between all the three groups. The results from the AMOVA showed that 36.1 % of the genetic variation in *B. lunaria* was partitioned among populations and 63.9 % within populations. The level of differentiation ($\Phi_{ST} = 0.361$) was highly significant ($p < 0.00001$).

The three individuals of *B. lunaria* were all allocated to Lungau in Austria ($p = 0.002$ for all individuals).

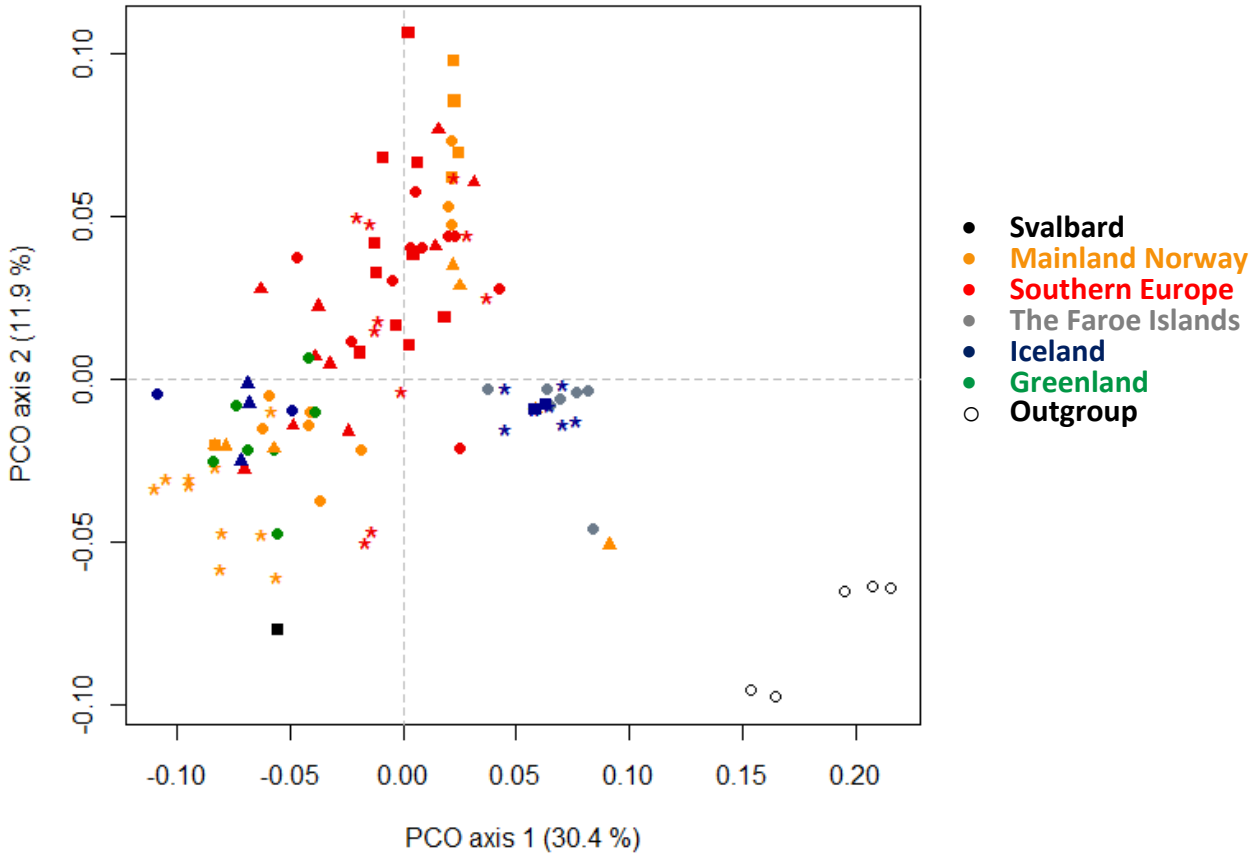


Figure 8. *PCoA plot of Botrychium lunaria made with Hamming distance measure.*

Black; square = Bockfjorden, **Orange**; stars = Finse, dots = Folldal, squares = Kåfjord, triangles = Tønsvikdalen, **Red**; stars = Bern, dots = Abruzzo, squares = Piemonte, triangles = Lungau, **Grey**; dots = Strendur, **Blue;** stars = Reykjanes, dots = Geysir, squares = Laugarvatn, triangles = Skaftafell, **Green**; dots = Tasiilaq, **Empty circles** = outgroup

3.3.2 *Sibbaldia procumbens* - genetic results

No genetic diversity was found within the Bockfjorden population, and it was only one genotype. The mean D value was 0.028, with values ranging from 0.000 (Bockfjorden) to 0.102 (Blasendalen in Greenland, n = 4). Low D values were found in Skaftafell in Iceland (D = 0.006, n = 11) and Aoste in Italy (D = 0.008, n = 10), whereas high D values were found in Kulusuk in Greenland (D = 0.046, n = 4) and Komi in Russia (D=0.043, n = 4) in addition to Blasendalen. (Table 1, Figure 12).

The population of *S. procumbens* from Bockfjorden had a DW-value of 103.7, which is lower than the average *S. procumbens* population (mean = 131.3). The populations that had the highest amount of rare markers were Komi in Russia (DW = 411.5, n=5), Blasendalen valley in Greenland (DW = 183.5, n=4) and Valais in Switzerland (DW = 173.9, n=10). The population with the lowest amount of rare markers was the tree individuals from Jan Mayen (DW=93.5), followed by Aoste in Italy (DW = 94.7, n=10) and then Tasiilaq in Greenland (DW = 96.1, n=10). (Table 1, Figure 12).

The PCoA plot of *S. procumbens* is shown in Figure 9. The first axis represented 54.5 % of the variation, the second axis 12.8 % and the third axis 5.6 %. The third axis did not provide any additional information to the first two axes. The PCoA plot showed a very clear separation of two groups within *S. procumbens* along the first axis, although a few individuals from Greenland (Blasendalen Valley and Tasiilaq), were located close to the middle. The first group contained the population from Bockfjorden in Svalbard, the mainland Norway populations and the Komi population from Russia. The second group contained all the populations from the Atlantic Ocean islands (except Svalbard) and the populations from Northern America. The outgroup, *S. cuneata*, was separated from *S. procumbens* along the second axis, and not along the first axis as might have been expected. To improve resolution of potential genetic structure within the focus species, the outgroup was removed from the PCoA plot (Figure 10). In this PCoA plot, the first axis represented 62.4 % of the variation, the second axis 6.7 % and the third 4.1 %. The third axis did not provide any additional information to the first two axes. Separation of the two groups still happened along the first axis. Some geographical structure was seen along the second axis, especially in first group (right), where the Russian population (Komi) was separated at the lower part. Populations within the second group (left) seemed to be a lot more intermingled. The neighbor-net (not shown) gave the same overall picture as the PCoA plot, but here the Bockfjorden individuals were located on the "shoulder" of the Komi-branch, (which again had individuals from Finse and Folldal on both sides). In addition, the biggest split was between *S. procumbens* and *S. cuneata*, which was not so apparent in the PCoA plot. The optimal number of clusters inferred from the Structure analysis confirmed the results from the PCoA and the neighbor-net, with two geographic groups (K = 2; not shown). The partitioning of the populations between the two groups was the same, with the Greenland populations showing a greater affiliation to the opposite group than the rest.

For *S. procumbens* 78.3 % of the genetic variation was partitioned among populations, while 21.7 % was partitioned within populations. The level of differentiation

(Φ_{ST} = 0.783) was highly significant (p<0.00001). AMOVA was also run with the two groups detected in the PCoA plot, the first group consisting of Svalbard, mainland Norway, the southern Europe populations and Komi in Russia and the second group including all islands in the Atlantic ocean, except Svalbard (The Faroe Islands, Iceland, Jan Mayen and Greenland), and the populations from Northern-America. The amount of genetic variation between these two groups was estimated to be 69.9 %, while the variation among populations within groups was estimated to be 15.7% and finally the amount of variation within populations was estimated to be 14.4%. All Φ-statistics were highly significant (p<0.00001), indicating substantial differentiation at all levels (Φ_{CT} = 0.699, Φ_{SC} = 0.522 and Φ_{ST} = 0.856).

In the allocation test, all the 25 individuals of *S. procumbens* were allocated to Folldal in mainland Norway (p = 0.049 for all individuals).

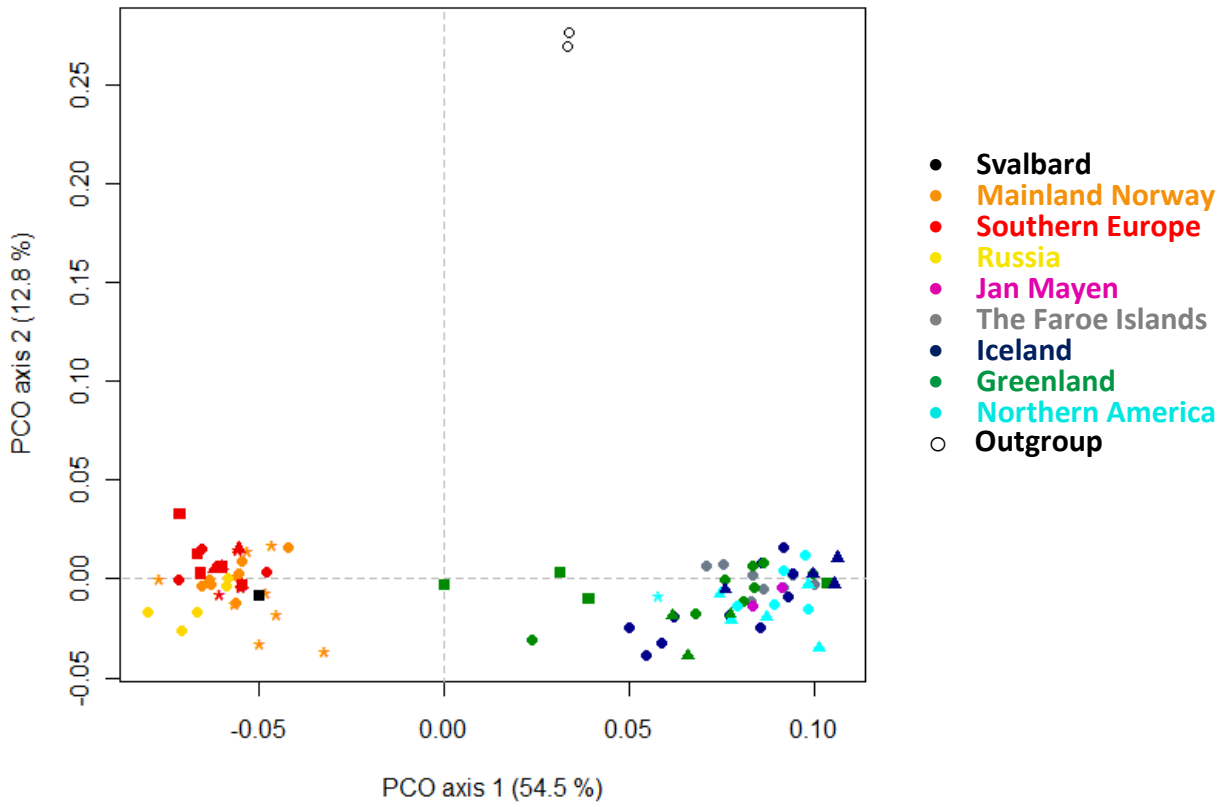


Figure 9. *PCoA plot of Sibbaldia procumbens made with Hamming distance measure.*

Black; **square = Bockfjorden,** Orange; **stars = Finse, dots = Folldal,** Red; **stars = Aoste, dots = Valais, squares = Piemonte, triangles = Lungau,** Yellow; **dots = Komi,** Purple; **dots = Jan Mayen,** Grey; **dots = The Faroe Islands,** Blue; **dots = Vestfirdir, triangles = Skaftafell,** Green; **dots = Tasiilaq, squares = Blasendalen Valley, triangles = Kulusuk,** Turquoise; **star = Nunavut, dots = Unalaska Island, triangles = Yukon,** Empty circles = **outgroup**

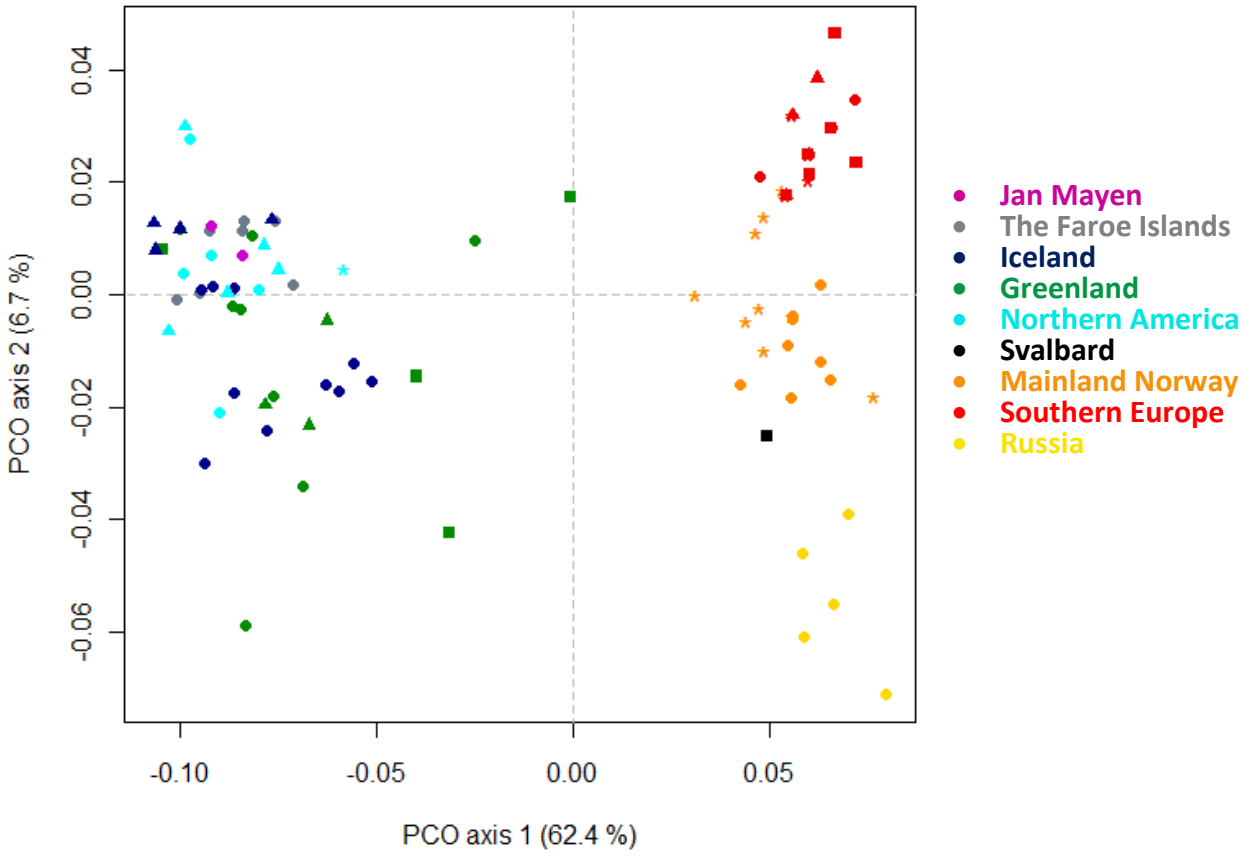


Figure 10. *PCoA plot of Sibbaldia procumbens, without the outgroup, made with Hamming distance measure.*

Black; **square = Bockfjorden,** Orange; **stars = Finse, dots = Folldal,** Red; **stars = Aoste, dots = Valais, squares = Piemonte, triangles = Lungau, Yellow; dots = Komi,** Purple; **dots = Jan Mayen**, Grey; **dots = The Faroe Islands,** Blue; **dots = Vestfirdir, triangles = Skaftafell,** Green; **dots = Tasiilaq, squares = Blasendalen Valley, triangles = Kulusuk,** Turquoise; **star = Nunavut, dots = Unalaska Island, triangles = Yukon**

3.3.3 *Kobresia simpliciuscula* ssp. *subholarctica* – genetic results

The populations of *K. simpliciuscula* ssp. *subholarctica* from Gipsvika and Blomstrand in Svalbard had D-values of 0.000 (n = 9 and 10 respectively). The population from Ossian Sarsfjellet had the highest D value among the Svalbard populations (D = 0.004, n=10), while the population from Flatøyrdalen had a D value of 0.002 (n=9). The populations of sub-species *K. simpliciuscula ssp. simpliciuscula* had D values of 0.0270 (Røros in mainland

Norway, n=5), 0.0240 (Lungau in Austria, n=3) and 0.0045 (Folldal in mainland Norway, n=8) as a comparison. Five genotypes/clones were found in total within Svalbard, two were exclusively for Ossian Sarsfjellet and two were exclusively for Flatøyrdalen. (Table 1, Figure 13).

As the sampling size of *K. simpliciuscula* ssp. *subholarctica* was too limited to calculate DW, the values were calculated together with the other subspecies *Kobresia simpliciuscula* ssp. *simpliciuscula. Kobresia simpliciuscula ssp. simpliciuscula* from Lungau in Austria was the population with most rare markers (DW=1052.2, n=3) - nearly ten times higher than the other *K. simpliciuscula ssp. simpliciuscula* populations (Røros in mainland Norway, DW =141, n = 5; Folldal in mainland Norway, DW = 123.5, n = 8). The four *K. simpliciuscula* ssp. *subholarctica* populations from Svalbard had DW values around 66.7. Gipsvika had the lowest DW value (DW = 64.3, n=9) and Ossian Sarsfjellet the highest value (DW = 69.5, n=10). The population from Flatøyrdalen had a DW value of 68.5 (n=9) and Blomstrand a DW value of 64.6 (n=10). (Table 1, Figure 13).

The PCoA plot of *K. simpliciuscula* ssp. *subholarctica* and *K. simpliciuscula* ssp. *simpliciuscula* is shown in Figure 11. The first axis represented 69.3 % of the variation, the second axis 20.3 % and the third axis 9.1 %. The first axis separated the population from Lungau and the outgroup equally far away from the rest, and the second axis separated the population from Lungau and the outgroup from each other. First along the third axis (not shown), was the Svalbard populations of *K. simpliciuscula* ssp. *subholarctica* separated from the mainland Norway populations of the other subspecies. The Svalbard populations were clustered close together, even covering each other in the PCoA plot. In the neighbor-net (not shown) it was maybe even clearer that the outgroup, *K. myosuroides* and the three individuals of *K. simpliciuscula* ssp. *simpliciuscula* from Lungau were equally distant from the rest. The Flatøyrdalen individuals clustered together, while several of the individuals from Ossian Sarsfjellet, Gipsvika and Blomstrand were intermingled. No further analyses of genetic structure were performed for *K. simpliciuscula* ssp. *subholarctica*.

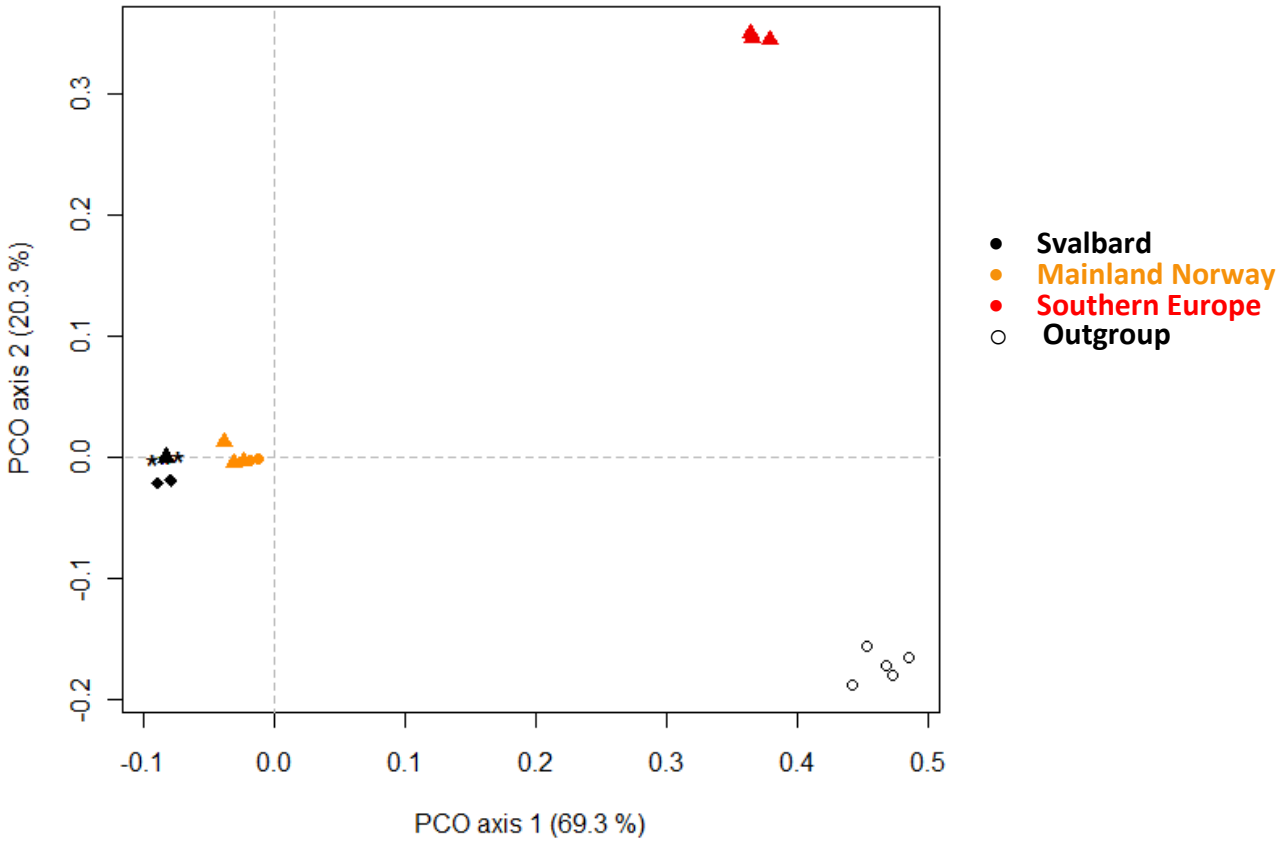


Figure 11. *PCoA plot of* ***Kobresia simpliciuscula ssp. subholarctica and K. simpliciuscula ssp. simpliciuscula.*** *Black; stars =* ***Ossian Sarsfjellet****, dots =* ***Gipsvika****, triangles =* ***Blomstrand****, diamonds =* ***Flatøyrdalen****, Orange; dots =* ***Folldal****, triangles =* ***Røros****, Red; triangles =* ***Lungau****, Empty circles =* ***outgroup***

3.3.4 *Ranunculus wilanderi* – genetic analyses

Ranunculus wilanderi from Kapp Thordsen in Svalbard had a low D value of 0.001 (n = 19) compared with the outgroup, *Ranunculus auricomus* (Folldal in mainland Norway, D = 0.021, n=5). The Kapp Thordsen population contained two genotypes.

Since *R. wilanderi* is only found in Kapp Thordsen Svalbard, it was not possible to calculate DW-values.

In the PCoA plot of *R. wilanderi*, the first axis accounted for nearly a 100 % of the variation and separated the outgroup from *R. wilanderi* as did also the neighbor-net (neither shown). No further analyses of genetic structure were performed for *R. wilanderi*.

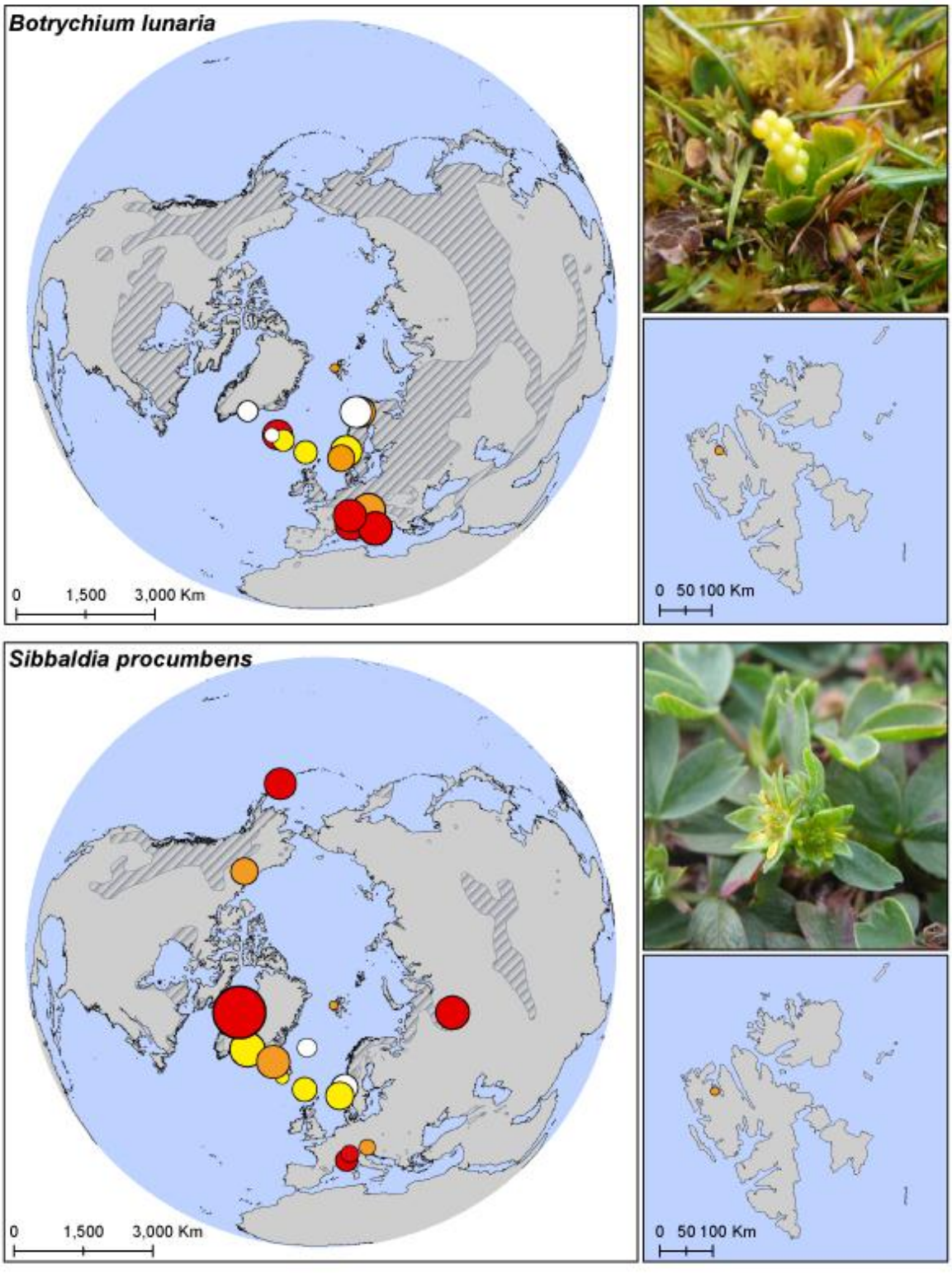


Figure 12. *Map showing the distribution of genetic diversity among all sampled populations of Botrychium lunaria and Sibbaldia procumbens. The size of the dots is proportional to the amount of gene diversity in the population. (Only exception is the Svalbard populations that contain no genetic diversity.) The color of the dots indicates the frequency of downweighted marker values for each population. Red represent the upper quartile, orange the middle high quartile, yellow the middle low quartile and white the lowermost quartile.*

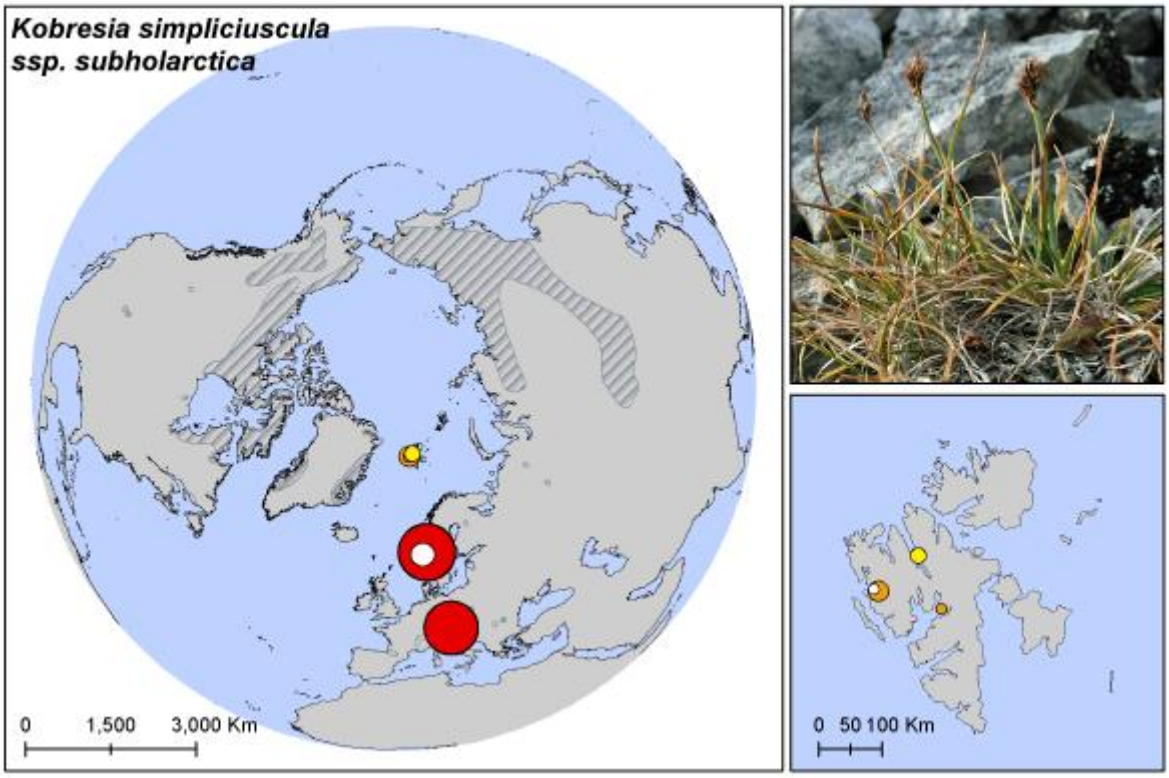


Figure 13. *Map showing the distribution of genetic diversity among all sampled populations of Kobresia simpliciuscula ssp. subholarctica and Kobresia simpliciuscula ssp simpliciuscula. The size of the dots is proportional to the amount of gene diversity in the population. (Only exception is the Svalbard populations that contain no genetic diversity.) The color of the dots indicates the frequency of downweighted marker values for each population. Red represents the upper quartile, orange the middle high quartile, yellow the middle low quartile and white the lowermost quartile.*

4. Discussion

4.1 *Botrychium lunaria*

4.1.1 Locality and population size

It is uncertain if the 21 shoots of *B. lunaria* found within 33 m^2 belong to one or several individuals. Based on the genetic investigations, the population probably consists of one genotype, indicating that it might be more appropriate to talk about ramets rather than individuals when estimating population size. The number of ramets is probably much higher than 21, considering that only the aboveground portion of the population was counted. A study by Johnson-Groh et al. (2002) showed that the density of the belowground individuals (gametophytes, juvenile sporophytes and dormant sporophytes) greatly exceeds the aboveground population in the genus *Botrychium*. The average ratio of belowground to aboveground plants was 332:1, based on eight *Botrychium* species. If *B. lunaria* is anywhere near this number, we can assume that the number of ramets in Bockfjorden is indeed higher than 21.

Aboveground *Botrychium* population numbers fluctuate considerably both between populations, and between years within the same population (Johnson-Groh 2002). Still it would have been interesting to compare the number of shoots found in this study with earlier findings. Unfortunately, no such numbers exists. Brattbakk discovered the population in Bockfjorden in 1974, but the number of shoots he found is unknown (Elvebakk & Spjelkavik 1981). However, in 1981, they visited the locality and reported that numerous specimens of *B. lunaria* were observed near the springs within at a very small area of about 10 x 20 meters (Elvebakk et al. 1994), which might be comparable to what we found. They also found another species of *Botrychium* that we did not find, *B. boreale*, which might indicate that they visited Bockfjorden in a good *Botrychium* year. Even though the population size is most likely larger than the 21 shoots counted in 2009, it probably consists of one single genotype and seems restricted to a very limited area.

4.1.2 Habitat, dispersal potential and possible threats to the population

The *B. lunaria* population was found growing in the warmest area in Bockfjorden. The small patch that it occupied was relatively damp with a lot of moss and vascular vegetation cover,

not unusual for its habitat elsewhere (Elven et al. 2007). The fact that *B. lunaria* seems to be restricted to the warmest site within a geothermal area indicates that temperature is a limiting factor for its distribution in Svalbard. In a study from Greenland, *B. lunaria* was in fact used as an indicator plant for mean July temperatures above 7° C (Karlsen & Elvebakk 2003), and in Svalbard it hardly reaches 6° C (WorldClim 2000). Its dispersal potential outside Bockfjorden, is therefore probably close to zero. The relatively high levels of bryophytes and cryptogamic crust might indicate relatively high nutrient levels, but since *B. lunaria* can be found growing on both nutrient poor and disturbed soil, this is probably of less importance (Stensvold 2008)

Moving over to the *B. lunaria*'s potential for spreading within the patch, no significant difference was found between control plots and focus species plots in the tests. But since the sample size was very low, it is not possible to rule out potential important differences. For instance, the control plots seemed to have more vascular vegetation cover. In the ordination performed for just the *B. lunaria* patch, the sample size was again probably too low to infer potential differences. Regardless, the green mossy habitat looked very homogenous in the field and in that case there could be potential for further population expansion. The problem is that we do not really know how much of the green patch the species actually occupy because of its subterranean nature, and there is a considerable chance that the species might already have been present in the control plot.

In the ordination performed for the whole Trollkjeldane area, the *B. lunaria* plots seemed to be distinct from most of the other plots due to high temperature and moisture levels in combination with a high cover of bryophytes and vascular vegetation. Nevertheless, some of the plots for the sedge *Carex capillaris*, (one of the focus species of the other study), were located in close proximity. *Carex capillaris* did actually co-occur with *B. lunaria* in the *B. lunaria* focus plots, and could be a good indicator plant of suitable habitat in the warmer parts of its distribution in the area. This sedge also seems to grow in areas with slightly lower moisture levels and less bryophyte and vegetation cover, which normally would suit *B. lunaria* (Jonsell & Karlsson 2000). Summed up, there might be some potential for population expansion for *B. lunaria* within the Trollkjeldane area. Although, we cannot know for sure that the species is not subterranean present in these areas already, or if there are undiscovered factors preventing it from further expansion.

The green mossy habitat close to the springs is very narrow and quickly shifts into a drier snowbed habitat just a few meters away from the springs. This can make the population especially vulnerable to human-caused and natural threats. Heavy grazing, trampling and

relocation of the springs were noted down as possible threats in the area. But then again, some of these threats might actually benefit the population. *Botrychium lunaria* is often associated with human-caused and natural disturbance, growing in avalanche meadows, talus slopes, recently deglaciated areas, pastures, along roadsides, airstrips and some areas of mining activity (Stensvold 2008). This feature of *B. lunaria's* ecology is probably a result of the species not being a very strong competitor. Its recent decline all over the world has been attributed to overgrowth caused by abandonment of pastures and artificial manuring (Jonsell & Karlsson 2000), and moderate grazing has been shown to increase its presence (Hellström et al. 2009). A moderate disturbance in Bockfjorden will therefore keep it from being wiped out by tougher competitors. The key to why *B. lunaria* is so resilient to disturbance is probably the belowground stages that provide *Botrychium* populations with a high degree of buffering against local extinction (Johnson-Groh et al. 2002). Still, if disturbance levels become too high, for instance if the associated hot spring dry out or shift location, this must also affect *B. lunaria* negatively in the longer run.

4.1.3 Conservation genetics

The fact that *Botrychium* species are expected to mainly reproduce via intragametophytic self-fertilization, makes the finding of only one genotype in Bockfjorden not very surprising. If *B. lunaria* historically is an inbreeding species, the lack of genetic diversity in Bockfjorden would probably be of less concern when it comes to inbreeding depression. However, my results indicate that most of the genetic variation can be found within the *B. lunaria* populations, not typically of a strictly inbreeding species. Furthermore, the populations of *B. lunaria* actually contain the highest levels of genetic diversity of all the focus species in this study. Although conflicting with most previous studies of the genus *Botrychium* (e.g. Soltis et al. (1988), Hauk and Haufler (1999)), high within population genetic diversity has also been found in newer studies on *Botrychium* (e.g. Camacho and Liston (2001), Williams (2012)). The reason why these newer studies, including mine, find more genetic variation within populations, might be due to the use of genetic fingerprinting techniques that yields a higher number of markers than the traditionally used isozymes (Camacho & Liston 2001). However, we all find that the differentiation between populations is relatively small. Does this mean that *B. lunaria* might be more outcrossing than previously thought? Not necessarily. Combinations of different life-history traits have the potential to influence the genetic structure of a species in various ways (Hamrick & Godt 1996), and the pattern observed in *Botrychium* can

probably be explained by a combination of high dispersal potential and a mating system that is mainly inbreeding (Camacho & Liston 2001; Stensvold 2008; Williams 2012). *Botrychium* spores are thought to mainly land in the immediate vicinity of the mother plant, being caught in vegetation etc. (Farrar 2006). However, if only 1 % of thousands of spores is transported over longer distances, this would still lead to a considerable amount of long distance dispersal. Furthermore, a mainly inbreeding mode of reproduction can be an advantage when colonizing new areas, since a new population can originate from only one individual with low likelihood of inbreeding depression. *Botrychium lunaria* is also a plant that often can be found growing in newly deglaciated areas and disturbed sites, further supporting its high colonizing potential (Stensvold 2008). A high colonizing nature, probably coupled with subsequent low divergence rates, may explain some of the rather odd geographic associations in my PCoA plot of *B. lunaria.* Stensvold (2008) found similar geographic associations in her PhD theses about European *B. lunaria.* For instance she found a mainland Norway-Iceland-Greenland line and a Norway-Iceland line.

If genetic variation within a population is caused by multiple colonization events, the evolutionary potential of the population would probably not be a problem. But only one genet was detected in Bockfjorden, indicating low genetic variation and consequently low adaptability to environmental changes. However according to Farrar (2006), *Botrychium* species rely heavily on their mycorrhizal fungal partner, and have therefore reduced their interaction with the environment to a minimum. The capacity to adapt to environmental changes is therefore largely transferred to the fungal partner, and the lack of genetic variation observed in Bockfjorden should in this context therefore not be of great concern.

Due to the low level of population differentiation, very few of the *B. lunaria* populations were especially divergent, and the Bockfjorden population was no exception. However, both the levels of genetic variation and the amount of rare markers seemed to decrease with distance from southern Europe, indicating that possible recolonization events after the last glaciation happened from a refugium in this area (also indicated in Stensvold (2008)).

4.2 *Sibbaldia procumbens*

4.2.1 Locality and population size

The continuous belt of *S. procumbens* was approximately 5 meter wide and 600 meter long, and estimated to consist of over a thousand plants. As for *B. lunaria*, there is a limited amount

of data available for comparison. The population of *S. procumbens* in Bockfjorden was discovered by Rönning in 1960, but he reported nothing about the number of plants found (Rønning 1961). The only documentation of population condition is from 1974 when all the plants were noted to be sterile (Kålås et al. 2010). This is a rather different observation from what we noted in 2009, when approximately 25 % of over a thousand plants were flowering. Even though there are no exact population size estimations of *S. procumbens* from before, it is believed that there has been an increase in the number of plants.

Although, it was over a thousand plants, they all shared the same genotype according to the genetic analyses. *Sibbaldia procumbens* possess the ability to reproduce by runners, but according to Coker (1966), this is extremely rare. Furthermore, the distribution of *S. procumbens* in the slopes above the springs was not *entirely* continuous, which might indicate that there must have been some seed recruitment. The thousand ramets or so is probably a result of self-fertilization, which is the supposed main mode of reproduction for *S. procumbens* in Svalbard (Brochmann & Steen 1999).

4.2.2 Habitat, dispersal potential and possible threats to the population

The snowbed habitat of *S. procumbens* in Bockfjorden was characterized by a relatively high vascular plant cover and a moderate bryophyte cover, which fits quite well with the general habitat description for the species. In his studies from northern Greenland, Raup (1969) found *S. procumbens* under similar conditions with a 61-90 % vegetation cover and relatively dry soil, like measured in this study. The most striking feature of the *S. procumbens* habitat was probably the differences in temperature and moisture levels found between focus species plots and control plots. The species obviously prefers sites that are situated in a more south-southeastern direction and that are (probably) consequently warmer. A difference in aspect could also be attributed to a preference for sites with maximum light intensity in a short growing season. For *S. procumbens* the association with higher temperatures is almost certainly of outermost importance for its existence in Svalbard. In Karlsen and Elvebakk (2003) study from East Greenland *S. procumbens* was used as an indicator plant of areas with a mean July temperature equal to, or higher than 6° C. As already mentioned, a mean July temperature of 6° C rarely happens in Svalbard and most certainly not so far north. Consequently, *S. procumbens* will probably not survive outside the favorable pocket created by the hot springs, and within this geothermal area, it seems dependent on sites with the most thermal energy. The focus species plots also seemed to be drier than the control plots. It is

uncertain if this was just a side effect of the general warmer temperature in the focus species plots or if *S. procumbens* had preferences for drier habitat. Anyway, it is noticeable that *S. procumbens*' possibilities for population expansion in its immediate surroundings might be relatively limited.

The ordination for the entire area showed that the habitat already occupied by *S. procumbens* share a combination of higher vegetation cover and lower temperature, moisture and pH levels than other sites within the Trollkjeldane area. Although *Carex capillaris* and *Tofieldia pusilla* seems to occupy relatively similar habitats, their environment seem to differ from the *S. procumbens* plots in important ways: The *T. pusilla* habitat seems to be situated in a cooler area, and the *C. capillaris* habitat seems to be situated in an area with higher moisture levels. If *S. procumbens* is not able to establish itself in cooler or moister sites in its immediate vicinity, chances of colonizing even moister of cooler sites are probably slim, and *S. procumbens* might have reached its optimum in terms of distribution both in its immediate surroundings and in the whole Trollkjeldane area.

When it comes to the threats noted in the field, two possible concerns were noted for the *S. procumbens* patch: Grazing and trampling. The area was most heavily grazed on the flat plains close to the hot springs, but grazing diminished as the terrain got steeper. According to Coker (1966), light grazing is just an advantage for the species. In the absence of grazing, shading by taller grasses and other herbs can smother the plant in more eutrophic habitats. However, he also stated that heavy grazing tends to eliminate *S. procumbens* because of slow leaf regeneration (Coker 1966). Like grazing, moderate trampling might also benefit the species. In a study of how trampling affected trail corridors in the north Rocky Mountain forests, *S. procumbens* was one of the species that was found to increase along the trails (Dale & Weaver 1974). The reason for this positive response to trampling is probably the same as for grazing, that disturbance might lower the competition from more vigorous species. However, if *S. procumbens* for some reason has suboptimal conditions in its habitat in Bockfjorden, the species might be more vulnerable to this kind of disturbance than normally presumed. Both grazing and trampling might be of larger concern in the flatter areas around the hot springs than in the steeper slopes and small inaccessible depressions where most of the *S. procumbens* plants are found.

A severe threat to the population not noted in the field is the predicted climate change. Snowbed specialists, like *S. procumbens*, risk losing their snowbed habitat due to earlier snowmelt in the future (Björk & Molau 2007; Galen & Stanton 1995; Schöb et al. 2009). The

species is adapted to maintain a metabolic "readiness" under the snow cover, a mechanism for monopolizing nutrient flushes and competitor-free intervals at snowmelt, and exploiting occasional long intervals for growth in years of little snow accumulation (Galen & Stanton 1995). If earlier snowmelt becomes the norm rather than the exception, the species will probably be outcompeted of invading plants that normally cannot exist under prolonged snow cover.

4.2.3 Conservation Genetics

Relatively low variation was observed in all the *S. procumbens* populations. However, the Bockfjorden population was the only population without any genetic variation at all. The low overall genetic variation was maybe somewhat surprising, due to the fact that *S. procumbens* is thought to only be self-fertilizing in some parts of its distribution range. Apparently is *S. procumbens* in the Alps and in Britain not self-fertilizing, but in Scandinavia and in Greenland it is (Coker 1966). But then again, *S. procumbens* is known to be pollinated by small insects, which also can lead to reduced amounts of variability within populations due to limited pollen movement and local foraging (Loveless & Hamrick 1984). The result from the AMOVA analysis, showing that most of the genetic variation was partitioned between populations, is then fitting quite well with both modes of reproduction.

The question is how this might affect the potential for inbreeding depression in the Bockfjorden population. Species with a mixed-mode of reproduction would probably experience quite high levels of inbreeding depression, since purging of deleterious alleles would not happen in the same way as in a strictly inbreeding species (Goodwillie et al. 2005). However, in both Norway and Greenland where the species is known to self-fertilize, it is quite common and seems to be in a healthy state. Based on the fact that the Folldal population from mainland Norway is closest relative to the Bockfjorden population, one should maybe expect that the lack of variation is of minor concern in this context. But according to Ellstrand and Elam (1993), the extent of inbreeding depression changes with the environment studied and may be more severe in competitive or otherwise challenging environments. For now, considering that the population in Bockfjorden probably has expanded, it might look as the conditions at least are okay. What is more uncertain is how well the Bockfjorden population might adapt to future changes in its environment. *Sibbaldia procumbens* also has a fungal partner (Elvebakk & Spjelkavik 1981) that might contribute to its adaptability, but it is unknown in what degree.

The genotype in Bockfjorden appears to belong to a Eurasian line, clearly distinct from a genetic line including Northern America and all the Atlantic islands. Within this group, *S. procumbens* did not seem to be especially divergent.

4.3 *Kobresia simpliciuscula* ssp. *subholarcitca*

4.3.1 Localities and population sizes

It has now been estimated that there are approximately 200 tussocks of *Kobresia simpliciuscula* ssp. *subholarctica* distributed over seven localities in Svalbard. The localities are situated in central to western parts of Spitsbergen, with two in the Kongsfjorden area, two in the inner Isfjorden area and three in the Wijdefjorden area. Mimerdalen is here omitted from the calculation, as *K. simpliciuscula* ssp. *subholarctica* has not been found there since 1925 (Inger Greve Alsos pers. comm. 1998, 1999, Kyle Hunter pers. comm. 2010). Although only some of the *K. simpliciuscula* ssp. *subholarctica* occurences have been visited in this study, the remaining sites have all been checked within the last ten years or so.

Around 80 to 90 of the tussocks were found in the Wijdefjorden area. In 2001, Elvebakk and Nilsen (2002) registered 50 tussocks of *K. simpliciuscula* ssp. *subholarctica* close to Lemströmfjellet. This is the second largest population of the subspecies in Svalbard. Although only three plants were found in this locality in 2011, they probably do not represent the same occurrence. Furthermore, Elvebakk and Nilsen (2002) also found a *K. simpliciuscula* ssp. *subholarctica* occurrence of 10-20 individuals in Reinsbukkdalen and an occurrence of 5 individuals in the neighbor valley, Flatøyrdalen. Nine additional plants were found in Flatøyrdalen in 2010 by participants of this project. The distance between Reinsbukkdalen and Flatøyrdalen is certainly not large, and since *K. simpliciuscula* ssp. *subholarctica* is wind pollinated, they probably cannot be called separate populations. The same might apply for other closely situated populations.

Elvebakk also discovered another *K. simpliciuscula* ssp. *subholarctica* population in Adolfbukta in the Isfjorden area in 2002 (Engelskjon et al. 2003). The number of specimens is not known, but the population is characterized as small by Engelskjon et al. (2003). The other occurrence in the Isfjorden area, Gipdalen, was visited in this study. The 20 tussocks was a doubling of the number that Engelskjøn found in 1985 (Bakken et al. 2006), although there might exists some differences in the way the plants were counted.

The highest number of *K. simpliciuscula* ssp. *subholarctica* individuals is found in Kongsfjorden. The two occurrences, Ossian Sarsfjellet and Blomstrandhalvdøya, are situated quite close to each other, and might not constitute two separate populations. The occurrence at Ossian Sarsfjellet was discovered in 1988, and the species was characterized as subdominant at the most (Elvebakk 1993). The 60 tussocks found in this study, is the first attempt to estimate the size of the occurrence. Together with the tussocks found at Blomstrandhalvøya, it is approximately 74 plants in the Kongsfjorden area.

Vegetative growth by rhizome is the dominant means of reproduction in *Kobresia* species, probably due to low seedling emergence caused by a harsh environment (Zhao et al. 2006). It is therefore not surprising that the approximately 200 *Kobresia simpliciuscula* ssp. *subholarctica* tussocks only consist of five genotypes. Again, it would be more appropriate to talk about ramets rather than individuals when estimating population size. The number of genotypes was highest in the largest occurrence at Ossian Sarsfjellet, and might reflect a higher level of sexual reproduction.

4.3.2 Habitat, dispersal potential and potential threats to the populations

In nearly all the locations (even the ones not visited in this study) *K. simpliciuscula* ssp. *subholarctica* was found growing in extremely rich fens, typical of its habitat also in other parts of the Arctic (Elvebakk 1993). The only exception was the more gravelly, but still moist, habitat observed at Blomstrandhalvøya. Apparently, *Kobresia simpliciuscula* (the species) can occasionally also be found in drier meadows and in plant communities at the transition of *Dryas* heath (Elvebakk 1993). However, its preference for extremely rich fens is so obvious that it is used as an indicator plant for the landscape type (Johnson & Steingraeber 2003).

In the vegetation map for Svalbard made by Elvebakk (2005), larger areas of calcareous fens are only found in the Isfjorden area. However, the subspecies has only been recorded from smaller occurrences of calcareous fens not visible in the vegetation map. Although there might be some dispersal potential within Svalbard, *K. simpliciuscula* ssp. *subholarctica* is probably limited by temperature. It seems to be relatively restricted to the warm sheltered areas in the warmest bioclimatic zone in Svalbard, which fits quite well with the fact that the subspecies has been used as an indicator plant for mean July temperatures of at least 6°C.

The subspecies might further have some potential for local dispersal in the two sites where ecological investigations were carried out (Gipsvika and Ossian Sarsfjellet). Even though the

tests and the ordinations revealed differences in vegetation cover and cover of bare ground in the two plot types, it was most certainly a result of *K. simpliciuscula* ssp. *subholarctica* being dominant in the focus species plots. Consequently, vegetation cover is higher in the focus species plots, and the amount of bare ground is higher in the unoccupied control plots. The fact that it was less room for cryptogamic crust in the K. *simpliciuscula* ssp. *subholarctica* plots, probably also account for the significant difference in this variable. In Gipsvika population expansion might be limited due to standing water. The difference between standing water and dripping wet would probably not be detected in the test due to the low sample size and the coarse moisture scale.

Grazing and trampling was noted as a potential threat to the *K. simpliciuscula* ssp. *subholarctica* occurrences. Although a bit far-fetched, *Kobresia* is reported to be preferred by livestock in Qinghai-Tibet plateau due to high levels of crude proteins and fats (Zhao et al. 2006). Apparently it is also a serious problem that *Kobresia* pastures become ecologically degraded due to over-grazing, trampling and human disturbance. I do not know if *K. simpliciuscula* ssp. *subholarctica* is preferred by geese and Svalbard reindeer, but it indicates that grazing and trampling might not be unproblematic. However, just moderate levels of grazing were detected at Ossian Sarsfjellet and only in the surroundings of the occurrence in Gipsvika.

Climate change might pose the largest threat to a wet-land species like *K. simpliciuscula ssp. subholarctica.* High Arctic wetlands are expected to be sensitive to global warming, since higher temperature might lead to changes in drainage conditions (due to melting of permafrost), evaporation rates and water supply (Young et al. 1997). In fact, in a study from the southernmost part of *K. simpliciuscula* ssp. *subholarctica's* distribution range (Montanta, U.S.A) the species was found to be in decline due to warmer temperatures and a drying of its habitat (Lesica & McCune 2009). So even though *K. simpliciuscula* ssp. *subholarctica* might be limited by colder temperatures elsewhere in Svalbard now, warmer temperatures might lead to its decline in the long run.

4.3.3 Conservation genetics

The level of genetic variation is extremely low in all Svalbard populations sampled for genetic analyses, and is probably also extremely low in the remaining populations not sampled. It has not been found any mature fruits of *K. simpliciuscula* ssp. *subholarctica* in Svalbard (Kålås et al. 2010) and it is uncertain in what degree the subspecies is reproducing

sexually within the archipelago. Most certainly it must have been reproducing sexually at one time, considering the scattered populations and the larger occurrences at Ossian Sarsfjellet and Lemnöfjellet. But several thermophilous species in Svalbard rarely set seeds under the present climatic conditions and may thus have a reduced recruitment of sexually produced offspring (Engelskjon et al. 2003). However, low levels of genetic variation were also found in the populations of the other subspecies, and D value in the population from Folldal was the same as for the Ossian Sarsfjellet population. The generally low overall levels of genetic diversity indicate that *Kobresia* species mainly reproduce clonally, and that low levels of genetic diversity are not uncommon. A mainly clonal mode of reproduction has also been found in a study of five *Kobresia* species from China, although levels of genetic diversity were much higher than observed here (Zhao et al. 2006). Although it seems to be a general feature in the *Kobresia* genus, it is a shortcoming that I have no material of *K. simpliciuscula* ssp. *subholarctica* from outside Svalbard, and therefore cannot compare levels of genetic diversity within the subspecies.

So how will this low level of genetic variation affect the potential for inbreeding depression? If reproducing mainly clonally by rhizome, new plants should be an exact copy of their parents, and subsequently it would be no increase in homozygosity. However in an asexual species, deleterious recessive mutations should accumulate since the offspring would inherit all the mutations of its parent (Muller's ratchet) (Pamilo et al. 1987). But since the species is not exclusively asexual, facultative sexual reproduction could have the potential to maintain levels of genetic diversity and long-term adaption (Zhao et al. 2006). The question is if this happens often enough in the Svalbard populations and if sexually breeding individuals are genetically different.

Unfortunately, it is not possible to infer how genetically distinct the populations of *K. simpliciuscula* ssp. *subholarctica* on Svalbard are from *K. simpliciuscula* ssp. *subholarctica* in other parts of the Arctic. I did not succeed in getting hold of material from outside Svalbard. In Svalbard, the population at Ossian Sarsfjellet seemed to be most genetically derived, but the difference between all populations was relatively small. Furthermore, the second largest population in Lemnöfjellet was not sampled. The PCoA plot confirmed that there was a division between the subspecies, but the *K. simpliciuscula* ssp. *simpliciuscula* population from Lungau was extremely different from all of the other populations. There is no voucher from this collection and there were only three individuals, so it remains a bit uncertain.

4.4 *Ranunculus wilanderi*

4.4.1 Locality and population size

The 51 individuals of *R. wilanderi* found in this study is probably a lower number than what was observed in 1992, when the population was characterized as "fairly extensive"(Elvebakk and Prestrud (1996), Reidar Elven pers. comm. 2012). However, a counting of *R. wilanderi* individuals was also performed in 2008, and together with the observations in this study, it has been observed four groups with a total of 85 individuals within a square kilometer (Kålås et al. 2010). But due to some uncertainties the number might be slightly lower (Inger Alsos pers. comm. 2012). No new finds of *R. wilanderi* has been made since it was discovered at Kapp Thordsen in 1871, which indicates that the number of undetected occurrences probably is low. Furthermore, it shows that the presence of *R. wilanderi* has been stable the last 140 years.

According to the genetic investigations, the *R. wilanderi* population only consists of two genotypes (or clones). It would therefore probably be more appropriate to talk about ramets, rather than individuals as above, when discussing the population size. A low number of genotypes were expected, since *R. wilanderi* most likely reproduce mainly by agamospermy. Furthermore, it means that the specimens of *R. wilanderi* that are kept ex situ in Tromsø botanical garden, is representative for most of the genetic variation contained within the Kapp Thordsen population. If the *R. wilanderi* ever should go extinct in the wild due to anthropogenic disturbance, it could still be reestablished artificially.

4.4.2 Habitat,local dispersal potential and possible threats to the population

Ranunculus wilanderi is restricted to a damp moss tundra in Kapp Thordsen. Some of the characteristics of the habitat, like the high levels of grazing, the high moisture level and the probably nutrient rich soil, are not unusual for a microspecies in the *R. auricomus* complex (Jonsell & Karlsson 2000). However, the moss tundra landscape type is rare both on a circumpolar scale and within Svalbard (Elvebakk 2005). This means that since *R. wilanderi* seems to be closely linked to this habitat type, its dispersal potential within Svalbard is probably very limited. Furthermore, taking into account that *R. wilanderi* was growing in a south-facing slope sheltered in the inner Isfjorden areas, the species is probably also limited by temperature.

Even though the species might have limited dispersal potential at a larger scale, local dispersal potential is probably higher. The control plots and focus species plots did not seem to differ much in the tests or in the ordination. Although the sample size (the number of plots) from the habitat was relatively low, the results appear plausible based on personal observations in the field. The moss tundra habitat seemed very homogenous, and the amount of unoccupied space by *R. wilanderi* was relatively high.

The biggest threat to the *R. wilanderi* population is probably that the moss tundra habitat might be vulnerable to climate change, in the same way as the *K. simpliciuscula ssp. subholarctica* wet land.

4.4.3 Conservation genetics

Although some genetic variation was found in *R. wilanderi*, the level was among the lowest measured in this study. The amount of genetic variation was also lower than that for the outgroup, an apomictic species also in the *R. auricomus* complex. If *R. wilanderi* mainly is reproducing by agamospermy, a low level of genetic variation is not surprising. Contrary to selfing, apomixis functions without meiosis and fertilization of egg cells, thus resulting in offspring with genotypes identical to that of the mother plant (Hörandl 2008). The little amount of genetic diversity that was actually found in the population, could be due to rare sexual events (Chapman et al. 2000; Van der Hulst et al. 2000), or new mutations (Paun et al. 2006). Studies have shown that apomicts in the *R. auricomus* complex often have partially aborted pollen, with the percentage of stainable pollen below 50 % (Horandl 1998), indicating that sexual reproduction is low. Furthermore, when sex becomes rare, so that few new recombinants occur, there should be a "runway" tendency for sex to be lost completely (Richards 2003). Nevertheless, a possible rare sexual event cannot be entirely ruled out.

In an apomictic species there will be no increase in homozygosity, which should lower the risk for inbreeding depression dramatically. However, the lack of recombination in an apomictic species will probably lead to accumulation of deleterious mutations (Muller's ratchet) (Pamilo et al. 1987) as might be the case for *K. simpliciuscula* ssp. *subholarctica*. Apomicts should therefore have low long-term evolutionary potential (Richards 2003). Nevertheless, since *R. wilanderi* is tetraploid the accumulation of deleterious alleles should take much longer time to reach a harmful level than for a diploid apomict. Furthermore, being a polyploid, *R. wilanderi* might possess genetic variation in the form of fixed heterozygosity (Brochmann & Steen 1999) that was not detected with the dominant genetic markers. In fact,

the species most certainly must be heterozygote, because it is aposporous and the apospory allele is simultaneously a recessive lethal factor (Horandl 1998). This means that even if the species might be threatened by accumulating deleterious mutations in the long run, it probably still possesses genetic variability which increases the evolutionary potential at a shorter time scale. Summed up, inbreeding and loss of evolutionary potential should be of minor concern if *R. wilanderi* truly is a tetraploid apomict.

The question if *R. wilanderi* represents an evolutionary divergent line becomes somewhat unnecessary, since there is only one population of *R. wilanderi* and it clearly constitute an own species. Although, the fact that *R. wilanderi* is a part of a complex where the number of apomictic socalled microspecies numbers over 600, tend to lower its uniqueness on a global scale. Nevertheless, this kind of apomictic speciation is an important source of biodiversity and in Svalbard *R. wilanderi* is the only representative of the *R. auricomus* complex. Closest relative, is probably a *Ranunculus auricomus* var. *glabrata* described from Novaya Zemlya and subsequently also identified from north-eastern Greenland (Engelskjon et al. 2003).

4.5 Implications for conservation

All the focus species have few and relatively small occurrences, which might make them especially vulnerable to anthropogenic disturbance. The most severe threat for all populations is probably the currently rising temperatures. *Ranunuculus wilanderi* and *K. simpliciuscula* ssp. *subholarctica* are closely linked to high-arctic wetlands, which are expected to become drier as the climate gets warmer, while *S. procumbens* risk losing its habitat due to an earlier snow melt. Furthermore, a geological study from Bockfjorden, shows that also the hot springs are sensitive to climatic changes. Apparently, the springs are most certainly dependent on discharge from the close by glacier, which means that if the glacier retreats, the hot springs also "retreat" (Haldorsen 2010). However, the springs will only dry completely out if temperature gets warmer than the Holocene optimum (Haldorsen 2010), and at that time will *B. lunaria* and *S. procumens* certainly not be dependent on the heat from the springs any more. Increasing goose populations, and subsequently more grazing is also linked to warmer temperatures (Kery et al. 2006). For *B. lunaria, S. procumbens* and *K. simpliciuscula* ssp. *subholarctica* might moderate grazing levels just be a benefit, but overgrazing will negatively affect them all. *Ranunculus wilanderi* is normally not grazed.

In this study I also looked at the genetic condition of all the focus species populations, since it might influence their ability to cope with human caused changes. All populations were found to contain no or low levels of genetic diversity. However, it should be mentioned that because AFLP is a dominant finger printing technique, heterozygosity cannot be truly measured, and genetic diversity might therefore be underestimated. But most plant populations in the flora of Svalbard contain little genetic diversity (Brochmann & Steen 1999), and the results found here is probably a reliable approximation to the truth. None of the species seemed to be especially prone to inbreeding depression, although evolutionary potential might be somewhat lowered.

For *B. lunaria*, the "Critically Endangered" category was maintained for the species on the regional red list, due to the extremely small population size and only one single occurrence. Furthermore, the few shoots seem to only to consist of one genotype. The species might have some dispersal potential within the warmest parts of Bockfjorden, but not in the rest of Svalbard. So the population will probably stay small. On a global scale, the *B. lunaria* population in Bockfjorden is probably mainly a curiosity, and only marks its northernmost occurrence. Within Svalbard, *B. lunaria* is maybe of highest conservation value when viewed as a part of a unique flora linked to the hot springs.

Sibbaldia procumbens was the only species that was moved over to a less threatened category (Endangered) in the regional red list. This was a result of a larger population size than previously thought and the observation of several fertile plants. Within Bockfjorden, the species seemed to be in relatively good state. Genetic investigations showed that the thousand plants in Bockfjorden only consisted of one genotype. It is uncertain how this might affect the species, but low levels of genetic diversity are also found in other parts of its distribution range where the species is more common. *Sibbaldia procumbens* has probably a relatively limited dispersal potential both within Bockfjorden and is the rest of Svalbard, due to a preference for higher temperatures. Like *B. lunaria,* is the *S. procumbens* population in Svalbard probably more of a curiosity on a global scale. But within Bockfjorden, *S. procumbens* is actually one of the most common plants in a very peculiar flora. The fact that Trollkjeldane nearly is a small "hot spot" for rare species should be of importance in conservation matters. Measures to conserve the flora in Bockfjorden could be monitoring of population sizes and levels of grazing/trampling etc.

Kobresia simpliciuscula ssp. *subholarctica* was kept in the category "Endangered" in the regional red list. It has several occurrences within Svalbard, which might indicate that it has been present within the archipelago over a longer time period and that it is an established

part of Svalbard's flora. Being closely linked to wet land, its occurrences should probably be monitored as temperatures get higher.

Ranunculus wilanderi is one of four endemic species for Svalbard, and is therefore of Norwegian responsibility (Bakken et al. 2006). Being restricted to only one locality with limited dispersal potential to other parts of Svalbard makes it vulnerable to unfavorable changes in its surroundings. Also being a species linked to wet-land, it should probably be monitored as the temperatures get warmer. The species was moved from the "Critically endangered" category to the "Endangered" category in the regional red list on the basis of the population size.

Monitoring these relatively warm loving species, might also tell us something about how the environment is changing. Although suffering from climate change on a longer scale, they would probably first increase in numbers if temperature truly is a limiting factor within Svalbard.

5. References

Bakken, T., J. Kålas, and Å. Viken. 2006. Norsk rødliste 2006 (The Norwegian Red List 2006). Artsdatabanken Norge.

Björk, R. G., and U. Molau. 2007. Ecology of alpine snowbeds and the impact of global change. Arctic, antarctic, and alpine research **39**:34.

Bonin, A., E. Bellemain, P. B. Eidesen, F. Pompanon, C. Brochmann, P. Bronken Eidesen, and P. Taberlet. 2004. How to track and assess genotyping errors in population genetics studies. Molecular ecology **13**:3261-3273.

Bråthen, K. A. 2009. More efficient estimation of plant biomass. Journal of Vegetation Science **15**:653.

Brochmann, C., and S. W. Steen 1999. Sex and Genes in the Flora of Svalbard- Implications for Conservation Biology and Climate Change. University of Oslo.

CAFF. 2010. Arctic biodiversity trends 2010 - selected indicators of change, Borgir.

Camacho, F. J., and A. Liston. 2001. Population structure and genetic diversity of Botrychium pumicola (Ophioglossaceae) based on inter-simple sequence repeats (ISSR). American Journal of Botany **88**:1065.

Chapman, H. M., D. Parh, and N. Oraguzie. 2000. Genetic structure and colonizing success of a clonal, weedy species, Pilosella officinarum (Asteraceae). Heredity **84**:401-409.

Coker, P. D. 1966. Sibbaldia Procumbens L. Journal of ecology **54**:823.

Dale, D., and T. Weaver. 1974. Trampling Effects on vegetation of the trail corridors of North Rocky Mountains Forest. Journal of applied ecology **11**:767-772.

Duchesne, P., and L. Bernatchez. 2002. AFLPOP: a computer program for simulated and real population allocation, based on AFLP data. Molecular ecology notes **2**:380.

Ehrich, D. 2006. A collection of R functions for handling AFLP data.

Eilertsen, O. 1990. Data manipulation and gradient length estimation in DCA ordination. Journal of Vegetation Science **1**:261.

Ellstrand, N. C., and D. R. Elam. 1993. Population genetic consequences of small population size: implications for plant conservation. Annual review of ecology and systematics **24**:217.

Elvebakk, A. 1993. Myrtust (Kobresia Simpliciuscula) på Svalbard. Polarflokken. Norsk Botanisk Forening, Tromsø.

Elvebakk, A. 2005. A vegetation map of Svalbard on the scale 1: 3.5 mill. Phytocoenologia **35**:951.

Elvebakk, A., R. Elven, S. Spjelhavik, D. Thannheiser, and H. J. Schweitzer. 1994. Botrychium boreale and Puccinellia angustata ssp. palibinii new to Svalbard. Polarflokken, Tromsø.

Elvebakk, A., and L. Nilsen. 2002. Indre Wijdefjorden med sidefjordar: eit botanisk unikt steppeområde. Rapport til Sysselmannen på Svalbard. University of Tromsø, Tromsø.

Elvebakk, A., and P. Prestrud 1996. A Catalogue of Svalbard plants, fungi, algae and cyanobacteria. Instituttet., Oslo.

Elvebakk, A., and S. Spjelkavik. 1981. Botanisering blant varme kjelder og vulkanar på nord-Svalbard. Polarflokken:104-113.

Elven, R., J. Lid, D. T. Lid, and T. Alm 2007. Norsk flora. Samlaget, Oslo.

Engelskjon, T., L. Lund, and I. G. Alsos. 2003. Twenty of the most thermophilous vascular plant species in Svalbard and their conservation state. Polar Research **22**:317-339.

Excoffier, L., G. Laval, and S. Schneider. 2005. Arlequin (version 3.0): an integrated software package for population genetics data analysis. Evolutionary bioinformatics online **1**:47-50.

Excoffier, L., P. E. Smouse, and J. M. Quattro. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics **131**:479.

Farrar, D. R. 2006. Systematics of moonworts *Botrychium* subgenus *Botrychium*. Ada Hayden Herbarium (ISC)

Iowa State University
Førland, E. J., and I. Hanssen Bauer. 2003. Past and future climate variations in the Norwegian Arctic: overview and novel analyses. Polar Research **22**:113.
Frankham, R. 2005. Genetics and extinction. Biological conservation **126**:131.
Frankham, R. 2010. Challenges and opportunities of genetic approaches to biological conservation. Biological conservation **143**:1919.
Frisvoll, A. A. 1978. Twenty-eight bryophytes new to Svalbard. The bryologist:122
Galen, C., and M. L. Stanton. 1995. Responses of Snowbed Plant Species to Changes in Growing-Season Length. Ecology **76**:1546-1557.
Goodwillie, C., S. Kalisz, and C. G. Eckert. 2005. The evolutionary enigma of mixed mating systems in plants: occurrence, theoretical explanations, and empirical evidence. Annual review of ecology, evolution, and systematics **36**:47.
Groom, M. J., G. K. Meffe, and C. R. Carroll 2006. Principles of conservation biology. Sinauer Associates, Inc., Sunderland.
Guillemaut, P., and L. Maréchal-Drouard. 1992. Isolation of plant DNA: A fast, inexpensive, and reliable method. Plant molecular biology reporter **10**:60.
Hald, M. 2004. Holocene paleoceanography and glacial history of the west Spitsbergen area, Euro-Arctic margin. Quaternary science reviews **23**:2075.
Haldorsen, S. 2010. Sensitivity to long-term climate change of subpermafrost groundwater systems in Svalbard. Quaternary research **73**:393.
Hammer, Ø., D. A. T. Harper, and P. D. Ryan. 2001. PAST: Paleontological Statistics Software Package for Education and Data Analysis. Palaeontologia Electronica **4**:9.
Hammer, Ø., B. Jamtveit, L. Benning, G,, and D. Dysthe, K, . 2005. Evolution of fluid chemistry during travertine formation in the Troll thermal springs, Svalbard, Norway. Geofluids **5**:140.
Hamrick, J. L., and M. J. W. Godt. 1996. Effects of life history traits on genetic diversity in plant species. Philosophical transactions - Royal Society. Biological sciences **351**:1291.
Hauk, W. D., and C. H. Haufler. 1999. Isozyme variability among cryptic species of Botrychium subgenus Botrychium (Ophioglossaceae). American Journal of Botany **86**:614.
Hellström, K., A. P. Huhta, P. Rautio, J. Tuomi, J. Oksanen, and K. Laine. 2009. Use of sheep grazing in the restoration of semi-natural meadows in northern Finland. Applied vegetation science **6**:45.
Hill, M. O., and H. G. Gauch. 1980. Detrended correspondence analysis: An improved ordination technique. Plant Ecology **42**:47-58.
Hoel, A., and O. Holtedahl 1911. Les nappes de lave, les volcans et les sources thermales dans les environs de la baie Wood au Spitsberg.
Horandl, E. 1998. Species concepts in agamic complexes: Applications in the Ranunculus auricomus complex and general perspectives. Folia geobotanica & phytotaxonomica **33**:335-348.
Hörandl, E. 2008. Evolutionary implications of self-compatibility and reproductive fitness in the apomictic Ranunculus auricomus polyploid complex (Ranunculaceae). International journal of plant sciences **169**:1219.
Huson, D. H., and D. Bryant. 2006. Application of Phylogenetic Networks in Evolutionary Studies. Molecular Biology and Evolution **23**:254-267.
IUCN. 2012. About biodiversity, International Union for Conservation of Nature. Available from (accessed January 2012).
Johnsen, K. I. E. i. C., B. Alfthan, L. Hislop, and J. F. Skaalvik. 2010. Protecting arctic biodiversity United Nations Environment Programme, GRID-Arendal.
Johnson-Groh, C., C. Riedel, L. Schoessler, and K. Skogen. 2002. Belowground distribution and abundance of *Botrychium* gametophytes and juvenile sporophytes. American Fern Journal **92**:80.
Johnson, J. B., and D. A. Steingraeber. 2003. The vegetation and ecological gradients of calcareous mires in the South Park valley, Colorado. Canadian Journal of Botany **81**:201.

Jónsdóttir, I. S. 2007. Terrestrial ecosystems on Svalbard: Heterogeneity, complexity and fragility from an Arctic island perspective. Biology and Environment: Proceedings of the Royal Irish Academy **105**:155.
Jonsell, B., and T. Karlsson 2000. Flora nordica. Bergius Foundation, Stockholm.
Jørgensen, M. H., R. Elven, A. Tribsch, T. M. Gabrielsen, B. Stedje, and C. Brochmann. 2006. Taxonomy and evolutionary relationships in the Saxifraga rivularis complex. Systematic botany **31**:702.
Kålås, J. A., Å. Viken, S. Henriksen, and S. Skjelseth. 2010. Norsk rødliste 2010 (Norwegian Red List 2010). Artsdatabanken, Norway.
Karlsen, S. R., and A. Elvebakk. 2003. A method using indicator plants to map local climatic variation in the Kangerlussuaq/Scoresby Sund area, East Greenland. Journal of Biogeography **30**:1469.
Kery, M., J. Madsen, and J.-D. Lebreton. 2006. Survival of Svalbard pink-footed geese *Anser brachyrhynchus* in relation to winter climate, density and land-use. Journal of Animal Ecology **75**:1172.
Kosman, E., and K. G. Leonard. 2005. Similarity coefficients for molecular markers in studies of genetic relationships between individuals for haploid, diploid, and polyploid species. Molecular ecology **14**:415.
Kruskal, J. B. 1964. Multidimensional scaling by optimizing goodness of fit to a nonmetric hypothesis. Psychometrika **29**:1.
Lande, R. 1988. Genetics and demography in biological conservation. Science **241**:1455.
Lande, R., and D. W. Schemske. 1985. The Evolution of Self-Fertilization and Inbreeding Depression in Plants. 1. Genetic Models. Evolution **39**:24-40.
Lesica, P., and B. McCune. 2009. Decline of arctic-alpine plants at the southern margin of their range following a decade of climatic warming. Journal of Vegetation Science **15**:679.
Liu, H. Y., T. Økland, R. Halvorsen, J. X. Gao, Q. R. Liu, O. Eilertsen, and H. Bratli. 2011. Gradients analyses of forests ground vegetation and its relationships to environmental variables in five subtropical forest areas, S and SW China. Sommerfeltia **32**:3.
Loveless, M. D., and J. L. Hamrick. 1984. Ecological determinants of genetic structure in plant populations. Annual review of ecology and systematics **15**:65.
Lydersen, C., H. Steen, and I. Alsos, Greve 2009. Svalbard - Miljøforhold og påvirkninger på rødlistearter. Artsdatabanken, Norge, Trondheim. Available from www.artsdatabanken.no (accessed 11. december 2011).
Nilsen, S. Ø. 2011. Miljøstatus i Svalbard (Environmental status in Svalbard). Norsk Polarinstitutt, Tromsø. Available from www.svalbard.miljostatus.no (accessed December 2012).
Økland, R. H. 2007. Wise use of statistical tools in ecological field studies. Folia geobotanica **42**:123.
Økland, R. H., and A. Edvardsen. 2006. Scale-dependent importance of environment, land use and landscape structure for species richness and composition of SE Norwegian modern agricultural landscapes. Landscape ecology **21**:969.
Økland, R. H., and O. Eilertsen. 1996. Dynamics of understory vegetation in an old-growth boreal coniferous forest, 1988–1993. Journal of Vegetation Science **7**:747.
Økland, R. H., and T. Økland. 2001. A Scandinavian perspective on ecological gradients in north-west European mires: reply to Wheeler and Proctor. Journal of ecology **89**:481.
Oksanen, J. 2011. Multivariate Analysis of Ecological Communities in R: Vegan.
Palmer, M. 2012. The Ordination Webpage. Oklahoma State University. Available from (accessed January 2012).
Pamilo, P., N. Masatoshi, and L. Wen-Hsiung. 1987. Accumulation of mutations in sexual and asexual populations Genetical research **49**:135-146.
Paun, O., J. Greilhuber, E. M. Temsch, and E. Hörandl. 2006. Patterns, sources and ecological implications of clonal diversity in apomictic Ranunculus carpaticola (Ranunculus auricomus complex, Ranunculaceae). Molecular ecology **15**:897.
Pritchard, J. K., M. Stephens, and P. Donelly. 2000. Inference of population structure using multilocus genotype data. Genetics **155**:945.

Qian, H. 2009. Understorey vegetation in boreal Picea mariana and Populus tremuloides stands in British Columbia. Journal of Vegetation Science **14**:173.
R Development Core Team 2011. R: A language and environment for statistical computing., Vienna.
Raup, H. M. 1969. The relation of the vascular flora to some factors of site in the Mesters Vig district, Northeast Greenland.
Richards, A. J. 2003. Apomixis in flowering plants: an overview. Philosophical transactions - Royal Society. Biological sciences **358**:1085.
Rønning, O. I. 1961. Some new contributions to the flora of Svalbard. Norsk Polarinstitutt, Oslo.
Schöb, C., P. M. Kammer, P. Choler, and H. Veit. 2009. Small-scale plant species distribution in snowbeds and its sensitivity to climate change. Plant ecology **200**:91.
Schönswetter, P., and A. Tribsch. 2005. Vicariance and dispersal in the alpine perennial Bupleurum stellatum L.(Apiaceae). Taxon **54**.
Serreze, M. C. 2000. Observational evidence of recent change in the northern high-latitude environment. Climatic change **46**:159.
Soltis, P. S., D. E. Soltis, and K. E. Holsinger. 1988. Estimates of Intragametophytic Selfing and Interpopulational Gene Flow in Homosporous Ferns. American Journal of Botany **75**:1765-1770.
Stensvold, M. C. 2008. A taxonomic and phylogeographic study of the Botrychium lunaria complex. Iowa State University
Sturm, M., J. Schimel, G. Michaelson, J. M. Welker, S. F. Oberbauer, G. E. Liston, J. Fahnestock, and V. E. Romanovsky. 2005. Winter biological processes could help convert Arctic tundra to shrubland. BioScience **55**:17.
The Svalbard Treaty. 1920. Treaty between Norway, The United States of America, Denmark, France, Italy, Japan, the Netherlands, Great Britain and Ireland and the British overseas Dominios and Sweden concerning Spitsbergen signed in Paris 9th February 1920.
Van der Hulst, R. G. M., T. H. M. Mes, J. C. M. Den Nijs, and K. Bachmann. 2000. Amplified fragment length polymorphism (AFLP) markers reveal that population structure of triploid dandelions (Taraxacum officinale) exhibits both clonality and recombination. Molecular ecology **9**:1-8.
Venables, W. N., and B. D. Ripley. 2002. Modern Applied Statistics with S. Springer, New York.
Vos, P., R. Hogers, M. Bleeker, M. Reijans, T. van de Lee, M. Hornes, A. Friters, J. Pot, J. Paleman, M. Kuiper, and M. Zabeau. 1995. AFLP: a new technique for DNA fingerprinting. Nucleic acids research **23**:4407.
Walker, D. A., M. K. Raynolds, F. J. A. Daniëls, E. Einarsson, A. Elvebakk, W. A. Gould, A. E. Katenin, S. S. Kholod, C. J. Markon, S. Evgeny, N. G. Moskalenko, S. S. Talbot, and B. A. Yurtsev. 2005. The circumpolar Arctic vegetation map. Journal of Vegetation Science **16**:267.
Walker, M. D., D. A. Walker, and J. M. Welker. 1999. Long-term experimental manipulation of winter snow regime and summer temperature in arctic and alpine tundra. Hydrological processes **13**:2315.
Whitlock, R., M. Hipperson, R. Mannarelli, K. Butlin, and T. Burke. 2008. An objective, rapid and reproducible method for scoring AFLP peak-height data that minimizes genotyping error. Molecular ecology resources **8**:725.
Williams, E. W. 2012. Exploring cryptic species in *Botrychium* s.s. ferns using genetics, morphology, and demography University of Wisconsin.
WorldClim. 2000. Global climate data. Museum of Vertebrate Zoology. Available from http://www.worldclim.org/ (accessed January 2012).
Young, K. L., M. K. Woo, and S. A. Edlund. 1997. Influence of Local Topography, Soils, and Vegetation on Microclimate and Hydrology at a High Arctic Site, Ellesmere Island, Canada. Arctic and Alpine Research **29**:270-284.
Zhao, Q. F., G. Wang, and Q.-X. Li. 2006. Genetic diversity of five Kobresia species along the eastern Qinghai-Tibet plateau in China. Hereditas **143**:33.
Ziegenhagen, B., P. Guillemaut, and F. Scholz. 1993. A procedure for mini-preparations of genomic DNA from needles of silver fir (Abies alba Mill.). Plant molecular biology reporter **11**:117.

Appendix

Box 1.Abbreviations:
pH = pH
slope = slope
temp3 = temperature at 3 cm
temp10 = temperature at 10 cm
moist = moisture level
vasc = vascular plant cover
bryop = bryophyte cover
lichen = lichen cover
crypto = cryptogamic crust cover
stones = cover of stones
baregr = cover of bare ground

Table A1. Correlations between explanatory variables - plots from the entire Trollkjeldane area

	pH	slope	aspect	temp3	temp10	moist	vasc	bryo	lichen	crypto	stones	baregr
pH		**0.009**	0.539	0.563	0.654	0.789	**<0.000**	**<0.000**	0.583	0.079	**<0.000**	**<0.000**
slope	**-0.245**		0.279	**0.008**	0.123	0.051	**0.010**	**0.005**	0.147	0.179	0.050	0.104
aspect	0.059	0.102		0.610	0.232	0.413	0.136	0.162	0.963	0.309	0.828	0.536
temp3	0.053	-0.242	-0.047		**<0.000**	**<0.000**	0.462	0.758	0.739	0.624	**0.008**	0.718
temp10	-0.041	-0.140	-0.111	**0.701**		**<0.000**	0.701	0.177	0.936	0.441	0.057	0.239
moist	-0.028	-0.205	-0.088	0.363	**0.444**		0.682	**0.006**	0.877	0.742	0.833	**0.001**
vasc	**-0.454**	0.239	0.141	-0.067	-0.035	0.043		**0.001**	0.604	0.057	**<0.000**	**<0.000**
bryo	**-0.478**	0.266	-0.134	-0.028	0.124	0.294	0.319		**0.026**	0.154	**0.001**	**<0.000**
lichen	-0.060	0.157	-0.005	-0.035	-0.009	-0.019	-0.056	0.244		0.681	0.477	0.304
crypto	0.174	-0.132	0.102	-0.047	-0.074	0.036	-0.186	-0.141	-0.047		**0.029**	0.262
stones	0.362	-0.194	0.022	0.259	0.186	0.024	**-0.456**	-0.321	-0.083	0.229		0.058
baregr	**0.489**	-0.174	-0.068	-0.038	-0.123	**-0.402**	**-0.504**	**-0.526**	-0.128	-0.127	0.217	

Right triangle = p-values ($p<0.05$ in bold), Left triangle = τ-values ($\tau>0.4$ and $\tau< -0.4$ in bold). For abbreviations see box 1.

Table A2. Correlations between explanatory variables - Botrychium lunaria

	pH	slope	aspect	temp3	temp10	moist	vasc	bryo	lichen	crypto	stones	baregr
pH		0.615	**0.010**	0.905	**0.031**	0.275	0.517	0.170	NA	**0.046**	0.252	NA
slope	-0.148		0.896	0.209	0.451	0.377	0.694	0.899	NA	1.000	0.885	NA
aspect	**0.772**	0.040		0.797	0.123	0.500	0.141	0.155	NA	0.086	0.767	NA
temp3	-0.071	-0.371	-0.077		1.000	0.275	0.365	0.533	NA	0.505	0.089	NA
temp10	**-0.643**	0.222	**-0.463**	0.000		0.127	0.897	0.105	NA	**0.046**	0.390	NA
moist	-0.357	-0.296	-0.231	0.357	**0.500**		0.365	0.124	NA	0.083	0.080	NA
vasc	0.197	0.123	**0.468**	-0.276	-0.039	0.315		0.602	NA	0.729	0.101	NA
bryo	-0.400	-0.038	**-0.432**	-0.182	**0.473**	**0.509**	0.161		NA	**0.044**	0.149	NA
lichen	NA	NA	NA	NA	NA	NA	NA	NA		NA	NA	NA
crypto	**0.655**	0.000	**0.589**	-0.218	**-0.655**	**-0.655**	0.120	**-0.667**	NA		0.4450	NA
stones	0.356	0.046	0.096	**0.774**	-0.267	**-0.624**	**-0.541**	**-0.454**	NA	0.2722		NA
baregr	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	

Right triangle = p-values (p<0.05 in bold), Left triangle = τ-values (τ>0.4 and τ< -0.4 in bold). For abbreviations see box 1.

Table A3. Correlations between explanatory variables - plots for Sibbaldia procumbens

	pH	slope	aspect	temp3	temp10	moist	vasc	bryo	lichen	crypto	stones	baregr
pH		0.857	0.123	0.528	0.620	0.220	0.111	0.084	0.316	0.612	0.476	0.341
slope	-0.034		**0.003**	0.469	0.135	0.164	0.091	0.584	0.893	**0.033**	0.341	0.874
aspect	-0.291	0.565		0.856	0.496	0.133	**0.019**	0.143	0.282	0.286	0.937	0.873
temp3	0.118	0.137	0.034		**<0.000**	0.488	0.110	**0.036**	**0.023**	0.612	**0.032**	0.874
temp10	0.092	0.281	0.129	**0.700**		0.364	0.202	0.111	0.082	0.782	**0.048**	0.634
moist	0.260	-0.299	-0.325	-0.148	-0.193		**0.041**	0.162	0.084	0.355	0.578	**0.041**
vasc	-0.303	0.326	**0.453**	-0.306	-0.244	**-0.447**		**<0.000**	**0.026**	**0.040**	0.338	0.471
bryo	0.328	-0.105	-0.283	**0.400**	0.303	0.305	**-0.682**		**0.006**	0.816	0.130	1.000
lichen	0.214	0.029	-0.234	**0.489**	0.372	**0.426**	**-0.489**	**0.605**		0.892	0.130	0.484
crypto	0.098	-0.417	-0.210	0.099	0.054	0.206	**-0.408**	0.046	-0.030		0.872	0.746
stones	0.153	0.207	0.017	**0.462**	**0.426**	-0.138	-0.212	0.333	0.377	-0.036		0.581
baregr	-0.207	0.035	0.035	-0.035	0.104	**-0.516**	0.161	0.000	-0.177	-0.074	-0.140	

Right triangle = p-values (p<0.05 in bold), Left triangle = τ-values (τ>0.4 and τ< -0.4 in bold). For abbreviations see box 1.

Table A4. Correlations between explanatory variables - Kobresia simpliciuscula ssp. subholarctica, Gipsvika

	pH	temp3	moist	vasc	bryo	crypto	stones_ground
pH		**0.036**	0.742	0.258	**0.047**	**0.039**	0.226
temp3	**-0.484**		0.061	0.484	0.574	0.833	0.526
moist	-0.087	**0.486**		**0.017**	0.117	0.117	0.282
vasc	-0.267	-0.161	**-0.633**		**0.033**	**0.023**	0.054
bryo	**-0.471**	0.130	**-0.418**	**0.504**		**0.005**	**0.012**
crypto	**0.487**	-0.049	**0.418**	**-0.538**	**-0.661**		0.100
stones_ground	0.286	-0.146	0.286	**-0.454**	**-0.593**	0.390	

Right triangle = p-values (p<0.05 in bold), Left triangle = τ-values (τ>0.4 and τ< -0.4 in bold). For abbreviations see box 1.

Table A5. Correlations between explanatory variables – Kobresia simpliciuscula ssp. subholarctica, Ossian Sarsfjellet

	pH	slope	aspect	temp3	temp10	moist	vasc	bryo	lichen	crypto	baregr	stones
pH		0.171	0.229	0.498	0.123	0.626	0.383	0.498	0.101	0.440	0.146	0.699
slope	-0.370		0.471	0.412	0.856	**0.028**	0.316	0.316	0.303	0.146	0.649	1.000
aspect	0.344	-0.197		0.153	**0.025**	0.369	0.610	0.919	0.135	0.542	0.083	0.839
temp3	0.073	-0.209	-0.385		**0.012**	0.361	0.787	0.417	0.610	0.281	0.087	0.369
temp10	**-0.409**	0.046	**-0.599**	**0.629**		0.732	0.590	0.719	0.155	0.601	0.072	0.862
moist	0.150	**-0.640**	0.279	0.263	-0.098		0.137	0.086	0.123	0.424	0.568	0.909
vasc	-0.233	0.256	-0.138	0.068	0.135	**-0.428**		0.928	0.052	0.858	**0.038**	**0.020**
bryo	0.181	-0.256	0.028	0.205	-0.090	**0.493**	0.023		0.838	1.000	0.787	0.072
lichen	**-0.470**	0.282	**-0.433**	0.138	0.381	**-0.478**	**0.523**	-0.055		0.684	0.083	0.222
crypto	-0.205	-0.368	-0.163	0.270	0.156	0.230	0.045	0.000	0.109		0.151	0.862
baregr	0.388	0.116	**0.468**	**-0.432**	**-0.449**	0.164	**-0.523**	0.068	**-0.468**	-0.360		0.106
stones	0.102	0.000	0.054	-0.225	-0.067	0.033	**-0.584**	**-0.449**	-0.327	0.067	**0.405**	

Right triangle = p-values (p<0.05 in bold), Left triangle = τ-values (τ>0.4 and τ< -0.4 in bold). For abbreviations see box 1.

Table A6. Correlations between explanatory variables –Ranunculus wilanderi

	pH	slope	aspect	temp3	temp10	moist	vasc	bryo	lichen	crypto	baregr	stones
pH		0.511	0.595	**0.010**	**0.017**	0.169	**0.042**	0.367	0.135	0.768	NA	NA
slope	0.177		0.722	0.232	0.602	**0.026**	0.457	0.315	0.484	0.844	NA	NA
aspect	0.158	-0.107		0.862	0.170	0.690	0.598	0.186	0.572	0.095	NA	NA
temp3	**0.660**	0.310	-0.050		0.155	0.078	0.205	0.280	0.051	0.923	NA	NA
temp10	**0.664**	-0.146	**0.426**	0.381		0.483	0.355	0.506	0.151	0.828	NA	NA
moist	-0.396	**-0.647**	-0.128	**-0.488**	-0.211		1.000	0.652	0.369	0.268	NA	NA
vasc	**0.537**	0.198	-0.154	0.322	0.254	0.000		0.552	0.693	**0.025**	NA	NA
bryo	0.256	-0.288	**0.419**	0.295	0.197	-0.138	-0.166		**0.019**	0.115	NA	NA
lichen	**-0.419**	0.198	-0.177	**-0.527**	**-0.420**	0.272	0.109	-0.699		0.209	NA	NA
crypto	0.083	0.056	**-0.522**	-0.026	-0.064	0.335	**0.618**	**-0.469**	0.369		NA	NA
baregr	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
stones	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

Right triangle = p-values (p<0.05 in bold), Left triangle = τ-values (τ>0.4 and τ< -0.4 in bold). For abbreviations see box 1.

Table A7. Correlations between explanatory variables and DCA axes – DCA for the whole Trollkjeldane area

	pH	temp10	temp3	moist	vasc	lichen	bryo	crypto	stones	baregr	slope	aspect
DCA1 (p)	**<0.000**	0.700	0.150	0.165	**<0.000**	0.427	**<0.000**	0.322	**<0.000**	**<0.000**	**0.001**	0.653
DCA1 (τ)	**0.582**	-0.035	0.129	-0.143	**-0.468**	-0.085	**-0.618**	0.096	0.368	**0.543**	-0.294	0.042
DCA2 (p)	0.766	**<0.000**	**<0.000**	**<0.000**	0.627	0.012	0.828	0.114	0.457	0.454	0.013	0.751
DCA2 (τ)	-0.027	**-0.504**	**-0.404**	-0.394	0.045	0.267	0.020	0.152	-0.073	-0.079	0.228	0.030

(p) = p-values (p<0.05 in bold), (τ) = τ-values (τ>0.4 and τ< -0.4 in bold). For abbreviations see box 1.

Table A8. Correlations between explanatory variables and DCA axes - DCA for Sibbaldia procumbens (with the focus species)

	pH	temp10	temp3	moist	vasc	lichen	bryo	crypto	stones	baregr	slope	aspect
DCA1 (p)	0.690	0.444	0.528	0.631	0.186	0.095	0.237	0.490	0.580	0.634	0.588	0.123
DCA1 (τ)	0.083	0.142	0.118	0.102	-0.251	0.356	0.224	-0.134	0.119	0.104	0.102	-0.291
DCA2 (p)	0.825	**0.006**	**0.019**	0.150	0.494	0.385	0.317	0.549	0.133	0.153	0.320	0.468
DCA2 (τ)	0.050	**0.510**	**0.437**	-0.306	-0.130	0.185	0.190	0.190	0.322	0.311	0.186	0.137

(p) = p-values (p<0.05 in bold), (τ) = τ-values (τ>0.4 and τ< -0.4 in bold). For abbreviations see box 1.